A Handbook of Practical Parasitology

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A HANDBOOK

OF

Practical Parasitology

BY

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London

JOHN BALE, SONS AND DANIELSSON, LTD.

OXFORD HOUSE

83-91, GREAT TITCHFIELD STREET, OXFORD STREET, W.

1910

JOHN BALE, SONS AND DANIELSSON LTD.
PRINTERS
83-91, GT. TITCHFIELD STREET, W.

TRANSLATOR'S NOTE.

The thanks of the Translator are due to several gentlemen for invaluable assistance and advice, especially to A. E. Shipley, Esq., F.R.S., Leonard Williams, Esq., M.D., J. Jackson Clarke, Esq., F.R.C.S., and F. W. Streatfeild, Esq., F.I.C., all of whom have done much to lighten the labours which the work entailed.

CONTENTS.

ERRATA.

Page 12, line 15, and elsewhere. *For* "plasma" *read* "plasm." (The terminal "a" has been added, both here and at intervals throughout the text, in error.)

,, 17, line 7. *For* "plasma" *read* "plasms."

,, 29, line 14 from end. *For* "of" *read* "or."

,, 29, line 11 from end. *For* "refuse" *read* "fuse."

,, 64, line 8 from end. *For* "violent" *read* "violet."

,, 68, line 8 from end. *For* "through the" *read* "through which the."

,, 104, line 4. *For* "cold saturated" *read* "cold-saturated."

,, 125, line 19, *should read:* "The Cysticerci (*Cysticercus fasciolaris*) of *Tænia crassicollis* are very distinct."

,, 137, line 24. *For* "armature and position" *read* "armature, position."

,. 142, line 4 from end. *For* "saline, which" *read* "saline, and."

,, 145, line 3 of footnote. *For* "oval" *read* "ova."

,, 152, line 17. *For* "develops" *read* "develop."

,, 157, lines 19 to 13 from end, *should read:* "present a bilateral arrangement. The shape of the embryonal sac is also very similar, being drawn out, in both species, into two long unbranched horns. The species are distinguished from one another by their length, which, in *M. denticulata*, is 40 cm. and in *M. expansa* is 4 to 5 metres; by the consequent difference in the time necessary for the development of the proglottides; and by the occurrence, in *M. expansa*, of inter-"

,, 164, line 24. *For* "Filaria" *read* "Filariæ."

,, 180, line 18. *For* "*Gamasidæ*" *read* "Gamasidæ."

,, 181, line 7. *For* "*Cytoleichidæ*" *read* "Cytoleichidæ."

,, 192, lines 14 and 15. *For* "*Cuticolæ*, the *Gastricolæ*, and the *Cavicolæ*. The" *read* "cuticolæ, the gastricolæ, and the cavicolæ. The."

A HANDBOOK OF

PRACTICAL PARASITOLOGY.

PART I.

PROTOZOA.

A.—GENERAL SURVEY.

I. INTRODUCTORY: UPON THE ORGANIZATION OF THE PROTOZOA.

THE Protozoa are single-celled animal organisms which may attain to comparative complexity of structure, owing to the fact that their body-parts vary according to the functions which they are required to perform. These modifications do not, however, affect the unicellular "form-value" of the organism, and for this reason the modified processes of Protozoa are termed "cell-organs" or "organelles," to distinguish them from the true organs, built up of many cells, of the Metazoa. The most important of these organelles are as follows:—

1.—*Organelles of Protection and Support.*

(i.) *Superficial Modifications.*—In the case of the greater number of the Protozoa a viscid, hyaline, outer or ectoplasm (ectosarc) is distinguished from a more liquid and granular inner or endoplasm (endosarc). In many parasitic varieties, however, and especially the cell-parasites, there is no differentiation of ecto- from endoplasm. In others, the difference is too slight to maintain constancy and distinction of form; hence the changes in shape of the Amœbæ. Stability of form is maintained by means of a thickened outer layer of plasm, and this is termed variously periblast (of Flagellates), cuticle (of Gregarines), and pellicle (of Infusoria and soil Amœbæ).

(ii.) *Axis-filaments* occur in many of the Flagellates. Their purpose is to maintain constancy of form. They are seen in *Trichomonas*, *Herpetomonas*, and in several of the Trypanosomes.

(iii.) *Protective secretions* of the protoplasm are frequently observed in the parasitic Protozoa in the developmental stage at which they

convey infection. They take the form of cysts or shells, the latter occurring in the cnidospores of the Myxosporides and their congeners. During the process of encysting, the formation of the true hard cyst is frequently preceded by the secretion of a casing of mucous or colloid matter. Propagation within the cyst may follow, but it is by no means the rule (compare, for instance, *Balantidium coli*).

2.—*Organelles of Movement.*

(i.) *Pseudopodia.*—Variously shaped processes thrown out by protoplasm, which are capable of slowly changing their form. They are projected and withdrawn, giving rise to the characteristic "amœboid movement," a kind of flowing or rolling progression.

(ii.) *Flagella* (Whips).—Long, fine, plasmic processes occurring in small numbers only. Like the tail of the spermatozoon, these flagella are furnished with an elastic axis-filament which serves as a support, and by means of which they are able to perform rapid waving movements, generally in a spiral direction.

(iii.) *Cilia* (Filaments).—Hair-like projections from the plasm, shorter than the flagella, and generally present in larger numbers. They are arranged in regular rows and move successively in a definite direction in waves. They are characteristic of the Ciliata. The amalgamation of several cilia in a cross-row gives rise to ciliated lamellæ or membranelles.

(iv.) *Myonemes.*—Contractile fibrillæ not unlike the muscle fibrillæ of the higher animals. They are arranged either lengthwise or across the body, and occur in the Flagellates, Gregarines, and Ciliates.

3.—*Organelles of Metabolism.*

(i.) *Special organs for the ingestion of food* are not present in those Protozoa which live in a medium containing nutrient matter, and which obtain food solely by endosmosis. Such are the Trypanosomes, Coccidia, and Gregarines. The greater number of the Protozoa, however, ingest solid food, and in these cases the pseudopodia, flagella, and cilia are employed to encircle the food-substance and whirl it within reach of the organism. Nearly all the Ciliates and a large number of the Flagellates possess, for purposes of ingestion, a special constant oral part (cytostome). In its simplest form it is an opening in the ectosarc, frequently continuing as a canal (cytopharynx) which penetrates a greater or less distance into the interior of the organism.

(ii.) *The food-vacuole* plays an important part in the digestion of solid material. The bolus of food is surrounded by a bubble-shaped liquid agglomeration, into which acids and ferments from the surround-

ing protoplasm are secreted, and these dissolve the assimilable constituents of the food-bolus. In certain species, however, this phenomenon may be absent (*Amœba blattæa*).

(iii.) *Defæcation,* the rejection of undigested food-remnants, is accomplished by means of a constant anal part (cytopyge). In the Flagellates and the Rhizopods, however, there is no fixed localization of the act of defæcation.

(iv.) *The contractile vacuole* is almost invariably present in the freshwater Protozoa, but is absent in most of the marine and in nearly all the parasitic varieties. With the exception of the *Opalina*, it is, however, generally present in the Ciliates. It pulsates regularly, becoming gradually filled and then suddenly discharging its contents, and is generally regarded as an organelle both of respiration and excretion. There are certain Amœbæ which possess a contractile vacuole, but which should not be included among the true parasites; they are to be regarded rather as saprophytic commensals.

The *nucleus* of the Protozoon is more varied in form and more complicated in structure than was formerly believed to be the case. There is frequently to be found within the nucleus a round inner body called the caryosome. This caryosome reacts more strongly to certain stains than do its surroundings, and in this way it is sometimes of practical service to the scientist. In colour solutions, for instance, the parasitic Amœbæ may be distinguished from the leucocytes by the shape of the nucleus, which, in the case of the former, is quite distinctive. It is important to remember that chromatic substances may be released from the nucleus, and may distribute themselves in smaller or larger fragments (chromides) in the plasm. Or, under certain conditions, the entire nucleus may break up into chromides. Such chromides are observed in the parasitic Amœbæ and others. In connection with the structure of the nucleus one other point requires mention. A functional double nucleation is frequently observed, which may be of two kinds. In the one case, one of the nuclei is a nucleus of metabolism, and the other a nucleus of reproduction (Infusoria). In the second case, somatic and generative chromatin are not separated, but are combined in both nuclei, while the division of function is such that it is customary to speak of a "principal" nucleus and a "locomotor" nucleus. An instance is provided by the Trypanosomes, the locomotor nucleus being here termed the "blepharoblast."

Propagation among the Protozoa is effected as follows:—

(i.) *By Division.*—The word may be used either in its narrower sense of a splitting into two, the daughter-organisms being of equal size; or it may be used in the sense of budding, the daughter-individuals being of different sizes.

(ii.) *By Multiple Division.*—The breaking up of the organism into a large number of daughter-cells. Under this heading are included :—

(*a*) *Schizogony.*—Multiple vegetative reproduction in the unencysted condition, the new individuals being known as merozoites. The process may be repeated several times.

(*b*) *Sporogony.*—A multiple vegetative reproduction which occurs once and without immediate repetition as the result of previous fecundation, and generally takes place within a cyst. The daughter-individuals are known as sporozoites.

(*c*) *Gamogony.*—Multiple reproduction of sexually immature individuals, the progeny, known as gametes, being sexually mature.

The combination in one species of several methods of propagation constitutes an *alternation of generation.*

Fecundation among the Protozoa consists in the merging of two nuclei and the production of a new homogeneous unit, known as the "new cell." It may take place in one of three ways :—

(i.) *Copulation.*—The merging of two distinct individuals (gametes) to form a new homogeneous individual. The sexually immature individuals are known as gametocytes. Their development into sexually mature, similar or dissimilar, gametes is the result of :—

(*a*) The reduction of the nucleus without division of the cell, as in the macrogametes of the malaria parasites and the Coccidia.

(*b*) Simultaneous multiple reproduction by gamogony, as seen in the Gregarines and in the formation of the microgametes of malaria parasites and Coccidia.

(ii.) *Conjugation.*—Here there is no merging of separate organisms, the meeting of the two conjugating individuals being quite transitory. They remain in contact only long enough to exchange portions of the nucleus, a free or male nucleus passing from each into the other, where it combines with a stationary, or female, nucleus to form the new cell. This method is characteristic of the Infusoria.

(iii.) *Autogamy.*—In this case the entire process of propagation is carried out by a single individual. The method varies in different species, but in all cases two gamete nuclei are produced which unite to form a single new cell (Amœbæ, Myxosporides).

II. General Remarks upon the Technique of Examining Protozoa.

The importance of using living Protozoa for purposes of investigation cannot be over-estimated. Without a knowledge of the motor phenomena, it is impossible to understand in its entirety the organization of certain species, such as the Amœbæ, Flagellates, and Infusoria. But, apart from this, there are certain peculiarities of structure which

either are not apparent in the preserved and coloured specimen, or are, at any rate, seen to much better advantage in the living object. It is true that the examination of living Protozoa is sometimes a matter of difficulty as, for instance, where the individuals are very much crowded together; where the plasm is exceptionally rich in light-refracting contents; or where opaque cyst- or shell-formations are present. But, if the correct diaphragm is used with the microscope, it is generally possible to obtain a view of the greater portion of the internal structure. Wherever fresh material is forthcoming, the living organism should always be examined first.

It is, however, expedient, and in certain kinds of scientific work it is indeed absolutely essential, to combine the two methods. Coloured specimens should be prepared and examined, after which the fresh material should again be studied. The knowledge gained by examination of the coloured specimens will show the living organism in a new light, and reveal the true significance of many details which at first escaped attention.

Wherever possible, parasitic Protozoa should be examined in their natural medium, *i.e.*, intestinal parasites in undiluted fæces or intestinal secretion; blood parasites in blood; and tissue parasites in lymph.

Should it be necessary to filter the liquid in which isolated parasites are to be examined, and especially where this liquid is obtainable only in very minute quantities (as the intestinal secretion of insects), the following method should be adopted: The preparation from which the liquid is to be obtained (in this instance a section of the intestine) is placed upon a morsel of filter paper on a cover-glass. The fluid rapidly filters through the paper and appears as a thin film upon the glass, to which the parasites to be examined are then transferred. Should the natural fluid be insufficient in quantity—and this is very frequently the case when examining the parasites of small insects, such as mosquitoes—it may be increased by the addition of normal saline solution. But it must be borne in mind that the addition of normal saline will shorten the life of the parasites. For this reason, it is sometimes better to increase the quantity of natural liquid by supplies drawn from the bodies of individuals of the same species as the host, the method of filtering above described being employed wherever necessary.

When studying the more active of the Protozoa (Flagellates, Ciliates) it will frequently be found necessary to restrict their motility to a certain extent. This is best done by increasing the viscosity of the medium in which they are to be examined by adding some colloid matter. The dried and bleached seaweed sold by druggists under the name of carragheen (it consists mainly of *Chondrus crispus* with a small quantity of *Gigartina mamillosa*), which, when soaked in water

swells up into a slimy colloid mass, is eminently suited to the purpose. The gradual thickening of the medium does not appear to harm the Protozoa, while its effect in retarding their movements not only facilitates observation, but in some cases is the sole means by which such is rendered possible. The best method is to put the seaweed into a bowl with some water, and when it has swelled to the consistency of a thick syrup, to introduce a small portion of the mass under the edge of the cover-glass. Where the Infusoria or Flagellates are not free-living but parasitic, the carragheen should be soaked in normal saline solution, serum, &c., instead of water. Another way is to add small portions of carragheen directly to the preparations, but in this case undissolved particles of weed are liable to get in the way and render observation difficult.

This method of reducing the activity of Protozoa is one which I invariably employ. I find that carragheen is more convenient to use and offers better results than cherry gum, gum arabic or gelatine. Statkewitsch[1] says that carragheen may be safely added to cultures of Protozoa. If this is done, however, the pieces of carragheen should first be washed in ½ to 1 per cent. solution of bicarbonate of sodium, they should be allowed to remain in the culture five to ten days, and at the end of that time all undissolved particles should be removed. After three to four weeks, the water in which the cultures are kept must be carefully changed, and at the end of a further three days fresh carragheen should be added. Paramœcia may be kept for months like this, without in any way suffering from the viscidity of the medium. The method possesses a certain analogy with that of the cultivation of Amœba upon solid media (see later).

Not the structure only, but the development of the Protozoa should be studied as far as possible in the living organism, the various stages presenting themselves to the student as a series of isolated but consecutive impressions. Care must be taken to prevent the medium in which the Protozoa are to be examined—and this applies particularly to the parasitic Protozoa — from becoming more concentrated by evaporation. The specimens should be prepared as rapidly as possible, and the edge of the cover-glass should at once be painted round with vaseline. Under these conditions the great majority of the parasitic Protozoa will remain alive for about two hours.

In addition to the usual microscopic methods, it is an advantage to study living Protozoa by means of drop cultures. For these it is necessary to use a glass slide which has been hollowed in the centre, or (though this is less satisfactory) a glass slide on to which a glass

[1] P. Statkewitsch, "Zur Methodik der biologischen Untersuchungen über die Protisten," *Arch. f. Protistenkunde*, vol. v, 1904, pp. 17-39.

ring is cemented. A small flat drop of the material to be examined is placed upon a cover-glass, which is then arranged upon the glass slide with the drop immediately over the hollow. To render this little chamber air-tight, a ring of vaseline is previously painted round the hollow in the glass slide, and into this the cover-glass is gently pressed.

Attempts to obtain pure cultures of Protozoa have frequently been made, but in the nature of things such experiments could only be successful in the case of species which are nourished entirely by endosmosis. But even among these, certain very delicate cell parasites, such, for instance, as the Coccidia, are not, as far as our present experience goes, susceptible of cultivation in artificial media. Up to the present, pure cultures have been unsuccessful, except in the case of the flagellate blood parasites (see later, under special heading). On the other hand, certain of the Protozoa which depend for their nourishment upon solid matter, such as the Amœbæ, may be fed with bacteria, and in this way cultivated in a form the practical value of which approximates very closely to that of the true pure culture (see later, under special heading).

The study of the more minute cytological details, and especially the structure of the nucleus, necessitates the colouring and fixing of the organisms. This may be done in the form either of sections or of cover-glass preparations. It should not be forgotten that, under certain conditions, fixing and colouring may have a diagnostic value. With regard to procedure, it is beyond the limits of this work to give more than an account of the methods which are of particular value in examining Protozoa. For all other information the student is referred to the text-books on the subject.

The most important fixing mixtures are the following:—

Alcoholic Solution of Mercuric Chloride (Schaudinn).—A mixture of 1 part absolute alcohol and 2 parts saturated solution of mercuric chloride. The latter ingredient is obtained by dissolving perchloride of mercury in boiling normal saline solution (0·75 gramme NaCl in 100 c.cm. distilled water) in such proportions that when the liquor cools a few crystals of perchloride of mercury are deposited. The proportion of mercuric chloride to alcohol need not be exact. I usually mix them by the eye in a test-tube. The mixture should be used warm (about 50° to 60° C.) and should take effect upon cover-glass preparations in about two to five minutes. Larger objects take proportionately longer. The specimens are then washed, first in 50 per cent., then 70 per cent. alcohol. They are next transferred to iodine alcohol, that is, 70 per cent. alcohol to which sufficient tincture of iodine or, better still, Lugol's solution (aqua destillata 100,

potassium iodide 6, iodine 4), has been added to make it the colour of port wine. Specimens are allowed to remain in this until they begin to turn a pale yellow (cover-glass preparations, about a quarter of an hour); they are then again rinsed in 70 per cent. alcohol, and are hardened (at least a quarter of an hour) in 80 per cent. alcohol, where, unless they are to be stained or embedded immediately, they should remain for future use.

Osmic Acid (Lee).—A mixture of 2 parts osmic acid in 100 parts of a 1 per cent. chromic acid solution should be kept in readiness as a foundation for Flemming's mixture and for the fixing of cover-glass preparations in osmium vapour. This latter method may be employed for blood parasites and Infusoria (see later, under special heading).

Acetic Acid Solution of Chromium and Osmium (Flemming).—One part of the above osmic acid mixture, 4 parts 1 per cent. chromic acid, 2 parts 1 per cent. acetic acid, 13 parts distilled water. This mixture should not be prepared until it is required for use. It may be employed with advantage for the preservation of blood containing parasites, for cover-glass preparations of certain Protozoa, as well as for small portions of organs containing parasites. It acts upon thin cover-glass preparations and upon blood (the latter should be allowed to drop into the solution) in ten to fifteen minutes; and upon pieces of organic tissue, which should be as small as possible, in half an hour to one hour. The specimens should be very carefully washed in distilled water, after which they are transferred to alcohol, the concentration of which is gradually increased. They are finally coloured with iron-hæmatoxylin or aniline dyes, or (though this is not so good) with ordinary hæmatoxylin or carmine.

Acetic Acid Solution of Chloride of Platinum and Osmium (Hermann).—Fifteen parts 1 per cent. platinic chloride solution, 1 part glacial acetic acid, 4 parts 2 per cent. osmic acid. To be used instead of Flemming's mixture and in the same way.

Acetic Acid Solution of Picric Acid and Mercurial Sublimate (Rath).—Equal parts of saturated solution of mercuric chloride (see alcohol sublimate) and picric acid (1 per cent. in distilled water), with the addition of ½ to 1 per cent. glacial acetic acid. Very useful for fixing portions of tissue containing parasites. Specimens should be left in the mixture for several hours, after which they should be washed, first in 50 per cent., and afterwards in 70 per cent. alcohol.

Picro-formol (Bouin).—Fifteen parts saturated watery solution of picric acid, 5 parts formalin (of commerce), 1 part acetic acid. This is said to produce very good results indeed, and is employed in the same way as picrin-sublimate.

Absolute alcohol is used to fix dry cover-glass preparations (see later, under Examination of Blood).

Parasitic Protozoa are fixed either in cover-glass preparations or in tissues from which sections are to be cut.

Cover-glass preparations should be made exclusively upon cover-glasses, as these are easier to manipulate in the later stages of preparation than glass slides.

Cover-glass preparations from organs are made by taking a portion of the organ to be examined in the forceps and passing the cut side, under gentle pressure, over the cover-glass.

Cover-glass preparations of bowel-contents can be made in a similar fashion by passing portions of the mucous membrane, or morsels of solid fæcal matter which are sufficiently firm to be held in the forceps, over the surface of a cover-glass.

Cover-glass preparations of fluids (blood, liquid bowel-contents, &c.) are made as follows: A small drop of the fluid to be examined is placed upon a cover-glass near the edge. A second cover-glass is placed at an acute angle, with one edge resting upon the first cover-glass, and in such a way that the liquid spreads itself in a long strip along the lower edge of the second cover-glass. By a movement of the second glass upon the first, at right angles to the edge at which both glasses touch, the fluid will be spread out in a thin layer upon the first cover-glass. It is important to remember that if the inclined cover-glass is moved in such a way as to push the fluid before it, the cellular elements are very liable to be injured by crushing. For this reason it is advisable always to move the inclined cover-glass away from the drop, so that it may draw the liquid after it. Kühne's cover-glass forceps will be found very convenient for this sort of work.

Cover-glass preparations should be fixed wet, and this applies to all subsequent stages of their technique. Dry-fixing is useful in the case of blood preparations only (see later, under Examination of Blood). In all other cases it is essential that the specimens should not be allowed to become dry, either before or after fixing, as the condition of preservation of the Protozoa is much impaired by drying. Specimens are wet-fixed as follows: The material to be examined is spread upon a cover-glass as thinly and evenly as possible, and the cover-glass is then placed, with the material downwards, in a watch-glass which has previously been filled with fixing mixture. There is sufficient albumen in blood and lymph, and, nearly always, in bowel contents and fæces, to coagulate under the influence of the fixing mixture (especially if the latter has been previously heated) and cause the material to adhere to the surface of the cover-glass. Should the medium in which the Protozoa are to be examined prove incoagulable, it will be impossible to prepare cover-glass specimens from it. Such a condition of things is, however, rare, and may be said to apply only to urine containing Flagellates. The process of fixing, like all other

details of preparation, should be carried out either in a test-tube, by the method described in the chapter upon Examination of the Blood, or under the cover-glass, the superfluous liquid being drawn off by means of filter paper from one edge, while fresh liquid is allowed to flow in at the other.

The cover-glass preparations when fixed are subjected to the same finishing processes (rinsing, hardening in alcohol, staining, transference to xylol and Canada balsam) as other specimens. The simplest and best method of storing them is in 70 to 80 per cent. alcohol in well-corked glass tubes, the inner diameter of which is slightly larger than that of the cover-glasses. The specimens are placed back to back in pairs and are kept from moving by means of a cotton-wool pad. If long tubes are used, several pairs may be arranged above one another. They should be separated by cotton-wool pads, and, for the sake of convenience, should be placed at right angles to one another.

The preparation of sections is essential, not only to a right understanding of the relationship of the parasite to the tissues of its host, but also to a proper appreciation of details of structure and development, many of which are not seen in the intact Protozoon, or only to a very imperfect extent. For the method of their preparation reference must be made to the various text-books of medical microscopy. There is one item of the technique, however, for which I propose to give detailed instructions, that, namely, of embedding, for the purpose of cutting single minute Protozoa into sections.

As a general rule, the Protozoa will be present in some numbers, and in that case it will rarely be necessary to arrange them in a given fashion before embedding. After fixing, the animalcules should be transferred to a small glass tube, where the various stages of the process should be carried out, including the spirit grades and the treatment with cedar wood oil (xylol or some other intermediary) to the final embedding in paraffin. It is better to centrifugalize each time before changing the liquid. Unless this is done, as, for instance, in the absence of a proper centrifuge, it will be necessary to wait until the micro-organisms have fallen to the bottom of the tube, and then carefully pour off the liquid, a glass rod being used to decant. As soon as the objects are soaked through with paraffin, the paraffin is rapidly cooled by plunging the glass tube into cold water. The tube may now be shattered, leaving a solid block densely packed with Protozoa which is ready for cutting.

Where a centrifuge is not available it is more convenient to follow an older method, recommended by Schaudinn. An arrangement similar to the micro-aquarium for the cultivation of Amœbæ, described on p. 15, is used, but with this difference. The cut in the glass slide should

be triangular in shape, and the two cover-glasses should be cemented to the slide with fish-glue. The specimens for embedding should be prepared in a watch-glass as far as the xylol stage, and then conveyed by means of a pipette into the micro-aquarium. The latter should be maintained in an upright position, and the organisms will be found to collect in the apex of the triangle. The xylol is now replaced by paraffin, and as soon as the objects have become saturated the glass slide is put into cold water. This hardens the paraffin and, at the same time, dissolves the fish-glue which keeps the cover-glasses in place, leaving free the triangular block of paraffin with the Protozoa clustering at its apex.

Schaudinn's method should be employed whenever it is desired to arrange single Protozoa (Infusoria, Gregarines) in such a way that a series of sections may be cut from them. But in order to do this, a material must be added to the xylol which will keep the single Protozoa in place and allow of their being controlled by the microscope. For this purpose, Schaudinn recommends the woolly palea of the young fronds and stems of the Java tree-fern, *Cibotium cummingii*, which is sold by druggists under the name of Penghawar-Djambie. It is an extremely fine-fibred felty substance, which may be easily cut when embedded in paraffin. After it is put into the micro-acquarium, a small groove is made in it with a blunt wooden point, and into this groove the object to be embedded is introduced.[1]

The best methods of staining are by the ordinary hæmatoxylin, Heidenhain's iron-hæmatoxylin, the Romanowsky stain, and the colouring of the living organism with neutral red.

Hæmatoxylin is the most valuable agent for staining the nuclei of Protozoa. The best form is undoubtedly Mayer's hæmalum, although the older alum-hæmatoxylin preparations may be used, the most convenient being Delafield's formula. The stain should be well diluted with distilled water or 1 per cent. alum solution, and cover-slip preparations and sections should be allowed to remain in soak for several hours; they may even be put in over night. They should be washed in running water and then examined under the microscope. If sufficiently stained, the specimens are then rinsed successively in 50 per cent., 70 per cent., 90 per cent., absolute alcohol in xylol, and afterwards mounted in Canada balsam, or in cedar wood oil which has been thickened to the consistency commonly used with an oil immersion lens. Canada balsam should be dissolved in xylol, and, when working with hæmatoxylin, it is expedient to use only those preparations

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[1] In their "Grundzüge der microscopischen Technik," 3rd Ed., p. 91, Lee and Mayer give a number of methods for placing small objects when embedding in paraffin. These are, however, somewhat more complicated than the method described above.

which are acid-free. (Grübler and Co., Leipzig, supply a Canada balsam which is quite acid-free.)

Should the specimens be too deeply stained, the superfluous colour may be removed by treating them with alcoholic solution of hydrochloric acid (a few drops hydrochloric acid to 100 c.cm. 70 per cent. alcohol). The process of decolorization should be watched under the microscope, and it should be allowed to proceed a little farther than at first appears necessary, as the colour deepens again when the specimens are blued in alkali. As soon as the desired shade is obtained, the decolorized specimens are plunged into ammoniated alcohol (1 drop of ammonia to 100 c.cm. 70 per cent. alcohol), when the colour will turn blue. They should afterwards be rinsed in clean 70 per cent. alcohol.

After the nuclei have been stained in hæmatoxylin, it may be found expedient to counter-stain the surrounding plasma in eosin or erythrosin. In this case, the specimens should be soaked, either in a concentrated watery solution of eosin before they are transferred to alcohol, or in a weak alcoholic solution of eosin after they are taken out of alcohol.

Of other stains, safranin is useful for staining objects which have been fixed by mixtures containing osmic acid. Borax-carmine and alum-carmine are frequently valuable, as, for instance, in establishing the presence of chromides. When using borax-carmine, the specimens should be decolorized with alcoholic solution of hydrochloric acid and afterwards rinsed in pure alcohol to free them from acid. Specimens stained with alum-carmine should be rinsed in distilled water.

Iron-hæmatoxylin (Heidenhain): Two and a half per cent. watery solution of iron-alum (ferrous ammonium sulphate) as a mordant and for purposes of differentiation; and a $\frac{1}{2}$ to 1 per cent. watery solution of hæmatoxylin (which should, if possible, be at least four weeks old) for purposes of staining. Cover-glass preparations and sections (the latter should not exceed 6 μ in thickness) should be laid in the mordant for four to twelve hours, or they may be put in over night. After careful rinsing in distilled water they are allowed to remain in the colour solution for six to twenty-four, or even, under certain conditions, thirty-six hours. They are then again rinsed in water and decolorized in the mordant, the precise degree of differentiation being carefully controlled by the microscope, which should be furnished with a strong dry lens. The specimens must once more be carefully rinsed, this time in running water for half an hour, and, after passing through the alcohol stages, they are transferred to xylol and, last stage of all, to Canada balsam or cedar wood oil.

The degree of differentiation will depend largely upon the purpose in view. As a general rule, however, the process should be continued

until the protoplasm is a pale grey in colour, to which the chromatic portions of the nucleus, stained a deep blue-black, offer a striking contrast. Very frequently, however (as in the Coccidia of rabbits), it is difficult to distinguish between the granulations of the plasm and the chromatic elements. In such cases it is advisable, after strong differentiation, to re-stain in a weak aqueous solution of Bordeaux red.

For details of the iron-hæmatoxylin method, as modified for the purpose of examining Trypanosomes, see chapter on Examination of the Blood.

The so-called Romanowsky or Giemsa stain is particularly prized for its brilliant colour-tints, but good results are only obtainable where the preparations are allowed to dry after staining. For this reason it is useful only in examining blood, and will be discussed later under that special heading.

Schaudinn states, however, that he has succeeded in using Romanowsky's stain for wet preparations. The specimens were strongly over-coloured, and the subsequent alcohol treatment, which was performed as rapidly as possible, sufficed to extract precisely the right amount of dye. I must admit that, personally, I have never been very successful with this method. No degree of over-colouring, however intense, would withstand the alcohol treatment necessary to dehydration, and the beautiful Romanowsky colouring of the nuclei was always destroyed.

Very varied effects are obtained by staining paraffin sections by Mallory's method. They are first immersed in a $\frac{1}{10}$ per cent. solution of acid fuchsin; then in 1 per cent. phosphormolybdic acid; and, finally, for five minutes in a solution consisting of aniline blue, 0·5 per cent., orange-green, 2 per cent., oxalic acid, 2 per cent., in distilled water. They are then rapidly washed in water, rinsed in 96 per cent. alcohol, and are transferred as soon as possible to absolute alcohol and xylol. By this method Vorticelli are coloured as follows: plasma, light orange; macronucleus, yellow; contractile vacuoles, brown; vacuoles of nutrition, blue; myonemes, bluish; muscle of stalk, dark blue; sheath of stalk, light blue; pellicle and cilia, orange; surface of the body, grey-blue. Myxosporides are coloured as follows: shells, orange; spore-plasma and nematocysts, violet; nucleus, red (O. Schröder). The differentiation is, however, somewhat capricious, and the method for this reason not to be unreservedly recommended.

The colouring of the living organism with neutral red is a useful method when studying certain more delicate structural arrangements, such as the organelles of metabolism. The stain must be very much diluted—a solution of 1 in 10,000 to 1 in 100,000 will be found active. If the Protozoon stains a bright red, acid is present; if the colour

is yellowish, it points to the presence of alkali. Neutral red may also be employed to distinguish between parasitic Amœbæ and leucocytes.[1]

B.—SPECIAL.

Class I. **Rhizopoda.**

Order: *Amœbina.*

Among the Rhizopods the only true parasites belong to the order Amœbina. It would be a serious oversight, however, to confine our investigations solely to the parasitic members of the group. Many body-cells—and this applies especially to the leucocytes—are capable of what is known as "amœboid" movement. Their possession of this power which, as its name shows, is to be regarded as a distinguishing characteristic of true Amœbæ, renders it necessary that the student should make himself acquainted with the salient features of the order, as exemplified in the free-living varieties. Only in this way can the knowledge be acquired which will enable him later to distinguish between the true parasites and body-cells which have the power of amœboid movement.

(*a*) *Free-living Amœbæ.*

Conditions under which Amœbæ may be Found.—If fresh hay or straw is put into a glass receptacle, covered with water, and allowed to stand for a time, numerous Protozoa will develop in the liquid. Among these, the first to attract attention will be the large and very active Infusoria, but Amœbæ, though frequently only the smaller forms, will also usually be found. The scum which appears upon the surface of the water, at first as a metallic film but later becoming thick and felty, should be first of all examined. Similar films are seen upon stagnant waters in the open air, and here Amœbæ are always to be found. They are also frequently present on the surfaces of the larger water-plants, as, for instance, the submerged portions of the leaves of *Stratiotes aloides*, or the undersides of the leaves of water-lilies. The larger varieties should be looked for in the vegetable slime of stagnant ponds. Amœbæ also occur in mossy tufts and in earth containing much humus (garden soil); there is, indeed, one variety which is known as the *Amœba terricola.* These organisms will appear if water is poured over the material before examination.

Cultivation of Amœbæ.—For anything in the nature of a minute study of the Amœbæ, it is of the first importance that the organisms

[1] *Cf.* S. Prowazek, "Vitalfärbung mit Neutralrot bei Protozoen," *Zeitschr. f. wiss. Zool.*, vol. lxiii, 1898, pp. 187-194, and "Zelltätigkeit u. Vitalfärbung," *Zool. Anz.*, vol. xxiv, 1901, pp. 455-460.

should be kept alive as long as possible. In many cases, further cultivation in small standing aquaria is all that is necessary. But sometimes it will be found expedient to isolate one or more individuals of a species in such a way that they may be kept constantly under observation. For this purpose, Schaudinn's micro-aquarium,[1] the construction of which is quite a simple matter, is very convenient.

A rectangular piece is cut with an emery-wheel out of one of the long sides of an ordinary glass slide. The excision should measure about 15 mm. by 10 to 14 mm. Over it should be cemented two cover-glasses sufficiently large to cover the opening completely; the size 21 by 26 mm., usually employed for mounting sections in series, will serve the purpose. The best cement is boiling Canada balsam, such as mineralogists use for mounting transparent sections. As a protective measure, narrow glass strips are cemented on to both surfaces of the slide, close to the two short edges. Water and animalcules should be introduced by means of a pipette, and the little aquarium may be placed horizontally as, owing to its capillarity, the water will not escape. A twig or two of green seaweed should be put in to keep the water fresh, or, with the micro-aquarium in a vertical position, fresh water may be added by means of a woollen thread from a vessel of water placed at a higher level. By varying the thickness of the glass slide and the size of the excision, the cubic contents of the aquarium can be regulated to suit the requirements of the particular organisms under observation. The micro-aquarium should be kept and studied in a damp chamber.

The study of Amœbæ is greatly facilitated by the fact that they are cultivable upon solid nutrient media. Pure cultures, in a bacteriological sense, are, it is true, impossible; but Amœbæ and bacteria may be cultivated together, the latter serving as food for the former. For this purpose any solid medium may be used, provided that it permits the bacteria a bare existence only, and does not offer conditions so favourable that they are able to overwhelm the Amœbæ. Many different media have been used with success, but a formula given by Frosch[2] deserves particular mention :—

Agar-agar	0·5 grm.
Ordinary alkaline bouillon	10·0 ,,
Tap water	90·0 ,,

Bouillon may be prepared by macerating freshly minced meat in

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[1] F. Schaudinn, "Ein Microaquarium, welches auch zur Paraffineinbettung kleiner Objekte benutzt werden kann," *Zeitschr. f. wiss. Microscopie*, vol. xi, 1894, pp. 326-329, with one illustration.

[2] P. Frosch, "Zur Frage der Reinzüchtung der Amöben," *Zentralbl. f. Bakt.*, part i, vol. xxi, 1897, pp. 926-932.

water for several hours (it may be left in all night) at a gentle heat, and then repeatedly sterilizing the resulting liquor.

Another very useful medium is prepared from carragheen. This medium is particularly useful in laboratories which are not provided with the sterilizing appliances necessary to bacteriological work. Its value is increased by the fact that, where the material employed is unaltered excreta, it is possible successfully to cultivate Amœbæ upon preparations of carragheen which have not been subjected to rigorous sterilization. Five to six grains of dried carragheen is washed in 1 per cent. soda solution and then boiled for half an hour in 100 c.cm. tap water.[1] The medium should be filtered through a clean cloth and when cold it will be ready for use. Colonies of mould-fungi or foreign bacteria should immediately be destroyed with a hot platinum needle.

It must be borne in mind, however, that not all Amœbæ can be cultivated upon solid media, and that the varieties which are parasitic in mammals have never been successfully grown outside the tissues of their host. I have, however, succeeded in cultivating on Frosch's agar medium, not Amœbæ only, but also Flagellates, obtained from the scum on hay or earth infusions.

Amœbæ are best cultivated in Petri's glasses. If the organisms are present in large numbers in the inoculation mass, they may be seen a few hours after inoculation following the bacteria and spreading out round the point of inoculation. They will be found, not only upon the surface, but within the substance of the medium, which, to facilitate observation under the microscope, should cover the bottom of the Petri glass in a very thin layer. In the course of the next few days the Amœbæ will increase in numbers and alter their distribution; cysts will form, also in largely increasing numbers. The cultures will remain active over a period varying from several weeks to several months.

There is yet another way of studying the living Amœba. A drop of the fluid which contains the organisms is placed upon a cover-glass, which is then turned, face downwards, upon a glass slide. The preparation should be allowed to stand for about ten minutes in order that the Amœbæ, which have contracted into balls on being disturbed, may again throw out processes. The movements may now be observed, and these will be found to vary considerably in different species. The *A. limax*, frequently found in infusions of straw, assumes a ribbon-like form as it flows forward; other Amœbæ, as for instance *A. proteus*,

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[1] *Cf.* M. Schubert, "Ueber die Züchtung der Amöben auf festen Nährböden," *Hygien. Rundschau*, 1897, No. 2; also E. Vahlkampf, "Beiträge zur Biologie und Entwickelungsgeschichte von *Amœba limax* einschliesslich der Züchtung auf künstlichen Nährböden," *Arch. f. Protistenkunde*, vol. v, 1905, pp. 167-220.

form more or less numerous finger-shaped pseudopodia; others again, especially *A. verrucosa*, adopt a more or less distinct rolling movement.[1] The movements of Amœbæ are influenced, moreover, by the medium in which the organisms are examined. On a colloid nutrient medium, for instance, they are slower than in water. The separation of the endo- from the ectosarc should be carefully noted, and the behaviour of both plasma during movement, as well as the regular action of the contractile vacuole, should be observed. Under favourable conditions it is sometimes possible to watch the ingestion of food particles by the process of "enfolding" and "drawing-in," as also the rejection of undigested food remnants. The nucleus, however, can as a general rule be studied in fixed and coloured specimens only.

The process of preserving Amœbæ can generally be carried out on the cover-glass. This is rarely possible, however, in the case of the soil Amœbæ, the superficial layer being here, as in the Infusoria, thickened into a close pellicle which prevents the organism adhering to the cover-glass. In the case of other species, a portion of the material containing the organisms (scum from straw or hay infusions should be triturated) should be transferred to a cover-glass, which is placed for ten minutes in a damp chamber. The cover-glass is then dropped, material downwards, into a small vessel containing hot alcoholic solution of sublimate. The cover-glass may now be rinsed, when it will be found that all extraneous matter and foreign organisms are washed away, while the Amœbæ remain upon the surface of the glass. This is explained by the fact that, during progression, Amœbæ secrete a sticky substance, which causes those individuals who have changed their position upon the surface of the glass, to adhere to it. Cover-glass preparations may similarly be made from Amœbæ cultivated upon solid media, by laying a cover-glass upon the surface of the medium, pressing it well down, and leaving it for several minutes. Preparations coloured with diluted hæmatoxylin, borax- or alum-carmine, or iron-hæmatoxylin, show with singular clearness the structure of the nucleus. In appearance it resembles a bubble, and it invariably contains a caryosome, which stains a darker colour than its surroundings. In many species, *A. limax* for instance, this caryosome is so large that a superficial observer might easily mistake it for the nucleus itself.

The study of the nucleus and the cysts, however, demands the preparation of sections. These are best prepared from agar cultures,

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[1] A detailed description of the movements of different Amœbæ is given in H. S. Jennings' "Contribution to the Study of the Behaviour of Lower Organisms," Washington, 1904; also L. Rhumbler, "Zur Theorie der Oberflächenkräfte der Amöben," *Zeitschr. f. wiss. Zool.*, vol. lxxxiii, 1905, pp. 1-52.

a small portion of the medium containing the organisms being cut out, fixed whole, and finished in the usual manner.

(*b*) *Parasitic Amœbæ.*

Among the Amœbæ parasitic in man, two varieties are entirely innocuous. They are of wide distribution and, where there is access to hospital material, readily obtainable.

(1) *Entamœba buccalis*, Prow., is found in the mouth, in the deposit upon the teeth under the gums, and especially in carious teeth. It occurs in most human beings, though generally in small numbers only. It is distinguished from the leucocytes by its larger size (6 to 32 μ), its higher refractive index, and the nature of its movements. The latter are performed by means of broad pseudopodia which are pushed out like a hernial sac. The living *E. buccalis*, moreover, when stained with neutral red, takes on a deeper colour than the leucocytes. There is marked differentiation between the

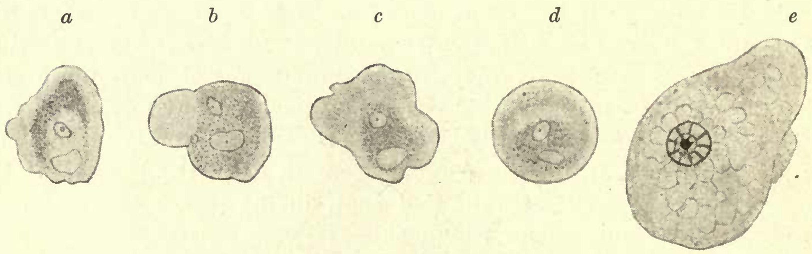

FIG. 1.—*Entamœba buccalis.* *a—d*, The same individual in four stages of movement, during an observation period of five minutes (magnified about 1,000 : 1); *e*, a fixed specimen coloured with iron-hæmatoxylin (magnified about 1,500 : 1). (From Braun, after Leyden and Löwenthal.)

hyaline ectoplasm and the nucleated endoplasm, the latter frequently containing numerous food boluses (fragments of leucocytes, bacteria, &c.). In the living organism, the nucleus, with its thick nuclear membrane and distinct caryosome, is frequently obscured by these food boluses. It is seldom possible to watch the process of reproduction (by simple fission), and the stages preparatory to encysting are even more difficult of observation.

E. coli (Lösch), Schaud., is found in most healthy people in the first part of the large intestine. Its numbers vary in individuals and localities, and it is apparently more numerous in the country than in the town. Its presence is demonstrated by the occurrence of characteristic round, eight-nucleated cysts in the fæces. During diarrhœa, which may be artificially induced, resting-cells and young cysts are passed. The movements of this Amœba can only be studied by

using a warm table. *E. coli* is distinguished from *E. buccalis* and the dysentery Amœbæ by its lower refractive index, and especially by the fact that, when motionless, there is no definite separation of the ecto- from the endoplasm. This differentiation is observed during move-

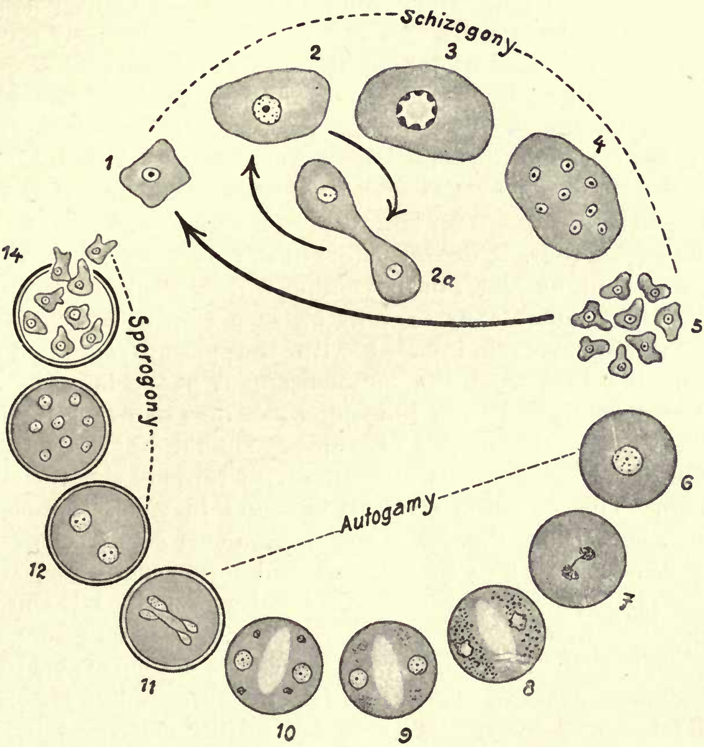

FIG. 2.—Diagram of developmental cycle of *E. coli* (Lösch), Schaud. (after Hartmann, slightly modified). *1*, Young Amœba. *2*, Adult Amœba. *2a*, Reproduction by fission. *3*, Multiple nuclear division; the chromatin has become aggregated into eight groups at the surface of the nuclear membrane. 4, Completed nuclear division. *5*, The Amœba has split up into eight daughter-individuals. *6*, Commencement of cyst-formation, *7*, First division of nucleus. *8*, Consequent incomplete cell-division and formation of chromides. *9*, Formation by the chromides of two pairing nuclei. *10*, Formation of two reduced nuclei by each pairing nucleus. *11*, Division of each of the reduced nuclei into a free (male) nucleus and a stationary (female) nucleus. *12*, By the merging of one free and one stationary nucleus, two new nuclei are formed. *13*, Adult cyst, containing eight daughter-nuclei, the result of repeated division of the new nucleus. *14*, Adult cyst, after entrance into the intestine of a fresh host, discharging the eight sporogenously produced Amœbæ.

ment, however, the pseudopodia proceeding entirely from the hyaline ectoplasm. This phenomenon is of use in distinguishing between *E. coli* and the leucocytes, the latter being devoid of ectoplasm. The nucleus can generally be seen in the living organism. It is round in shape, contains much chromatin, and is furnished with a thick

membrane and large caryosome. Isolated developmental stages may be observed, but it is impossible to follow the entire process, which is very complicated, without long and tedious study. The life-history of this organism is shown in fig. 2. It multiplies by simple fission and by schizogony. The splitting up of the nucleus during schizogony is very characteristic. The chromatin collects in eight portions upon the surface of the nucleus, and from these simultaneously proceed, the eight daughter-nuclei of the eight new organisms (fig. 2, *3—5*). As the fæces tend to become solid, in addition to dead Amœbæ, young cysts will be found. These originate in a casing of mucous matter which forms round the ball-shaped Amœba and which is followed at a much later stage by the true hard cyst. Changes of the nucleus, leading to self-propagation (autogamy), may be observed in the young mucous cysts (figs. 2, *6—12*), while in the older hard cysts the successive stages of nuclear multiplication, which result in the formation of the eight daughter-cysts, may be seen (fig. 2, *12—13*). Cats as well as man may be infected with the mature encysted Amœbæ, which, when they reach the commencement of the large bowel in the fresh host, burst and allow the young parasites to escape (fig. 2, *14*).

The intestinal Amœbæ of animals should be studied and their structure and development compared with those of the varieties found in man. The *E. muris* (Grassi) is found in small numbers at the commencement of the large bowel in about 50 per cent. of mice. It is closely related to the *E. coli* and forms eight-nucleated cysts. In the resting-state, however, the Amobæ reproduce themselves, not by multiple division of the nucleus and plasm, but by simple fission. Like the intestinal Amœbæ of man, they are not cultivable in artificial media. The cysts of another variety of Amœba, however, are found in the fæces of mice, and these are readily cultivable by smearing a small portion of the fæces upon the agar medium described above. This organism is not parasitic ; its cysts pass unchanged out of the bowel of the mouse, similarly to *Chlamydophrys enchelys* (Ehrbg.), as described by Schaudinn.[1]

In the terminal portion of the intestinal canal of frogs, an Infusorian, *E. ranarum* (Grassi), is found, generally in small numbers, which resembles the *E. coli*.

In the terminal portion of the intestinal canal of lizards (where, later, we shall find Flagellates) two varieties of Amœba, which have no relationship to the parasitic Amœbæ of man, are found. They are readily cultivated upon the agar medium, and will repay the trouble of observation. The one species, which is distinguished by its double

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[1] *Cf.* C. M. Wenyon, "Observations on the Protozoa in the Intestine of Mice," *Arch. f. Protistenkunde*, supp. i, 1907, pp. 169-201.

nucleus, is known as *Amœba diploidea*;[1] while the second variety has one nucleus only, and is apparently related to *A. limax*.[2]

A good deal of information may be gained from the study of *A. blattæ*, which is found in the terminal portion of the intestinal canal of cockroaches. This organism belongs to yet another group of Amœbæ and is particularly instructive on account of the very distinct "streaming" movements of the plasm. It should be sought in freshly caught beetles only, as after the beetle has been in captivity for two or three days the organisms disappear from the intestine. The head and hinder part of the body should be cut off, and the intestine may then be withdrawn with the tweezers from the hinder orifice.

Material for the examination of the dysentery Amœbæ of man is sometimes difficult to procure. The species most frequently met with is *E. tetragena* which is frequently found in people returning from hot climates.

(1) *E. tetragena* has only quite recently been isolated from *E. histolytica*. It is found in Africa and South America, and is recognized by the fourfold nucleation of its cysts. In its method of development it closely resembles the *E. coli*, but differs from it not only in the fourfold nucleation of the adult cysts, but also in possessing during the resting stage a homogeneous and highly refractive ectoplasm. In this latter property it resembles *E. buccalis* and *E. histolytica*. The ectoplasm is easily distinguished from the endoplasm, which has generally a lower refractive index and contains nuclei, vacuoles, food remnants, &c. The nucleus is very characteristic and quite different from that of the other bowel Amœbæ of man. Like the nucleus of *E. coli*, it is round in shape, contains a somewhat large amount of chromatin, and has a central caryosome, which may be large or small. When carefully stained, this caryosome shows a central spot which Hartmann[3] compares to the centriole in the centrosome of Metazoa. "Sometimes this centriole occupies the whole of the centre of the nucleus. In that case it is surrounded by a clear space, while the original margin of the caryosome persists as a sort of membrane." Reproduction during the inactive stage has been observed only in the form of fission. This species, which is not seen in man except in cases of dysentery, may be transferred to the cat, where it approximates very closely to *E. histolytica*.

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[1] M. Hartmann and K. Nägler, "Copulation bei *Amœba diploidea* n. sp. mit Selbständigbleiben der Gametenkerne während des ganzen Lebenscyclus," *Sitzber. Ges. naturf. Frde.*, Berlin, 1908, No. 5, pp. 112-125.

[2] M. Hartmann and S. Prowazek, "Blepharoblast, Caryosom u. Centrosom." *Arch. f. Protistenkunde*, vol. x, 1907, pp. 314-315, fig. 4 f.

[3] M. Hartmann, "Eine neue Dysenterieamöbe, *Entamœba tetragena* (Viereck), syn. *Entamœba africana* (Hartmann)," Beihefte z. *Arch. f. Schiffs- u. Tropenhyg.*, 1908, Beih. 5 (Verhandlg. d. deutsch. tropenmed. Ges. I.), pp. 117-127, fig. 9.

(2) *E. histolytica*, the dysentery Amœba of man, occurs in Egypt and Southern and Eastern Asia. It resembles the *E. tetragena* in having a well-developed ectoplasm, but it is to be distinguished from it and from the other Amœbæ which are found in the intestine of man by the structure of the nucleus in the resting stages. The nucleus of *E. histolytica* has no surrounding membrane and contains very little chromatin; for this reason it is rarely seen in the living organism. It contains, however, a small caryosome, and in this conforms to the general structural scheme of the amœboid nucleus. *E. histolytica* differs from all other parasitic Amœbæ in the nature of its permanent stages. Usually the entire amœboid organism encysts and the shell is readily discoverable among the fæces. In the case of the *E. histolytica*, however, the cyst is a minute structure sloughed from the surface of the organsim, and is extremely difficult of detection.

It is evident from the foregoing how important is the part played by nuclear structure in distinguishing the different species of Amœbæ, not only from one another, but, what is of even greater importance to the parasitologist, from body-cells such as the leucocytes (fig. 1, *e*). The student should seek every opportunity of making himself acquainted with the structural details of the Amœbæ, and for this purpose the varieties which are indigenous and easily obtainable, furnish excellent material.

Class II. **Neosporidia.**

Although many of the parasitic Protozoa closely resemble certain free-living forms, there are, nevertheless, a large number of varieties which possess no such resemblance. These are usually classed in a special group as Sporozoa. But with a greater knowledge of the subject, it has become increasingly doubtful whether these so-called "Sporozoa" are possessed of any attribute in common other than their adaptation to parasitism. In all other directions, they differ from one another so markedly that it has been found necessary to divide them into at least two groups. Of these, one, the Telosporides, appears to possess a certain distant resemblance to the Flagellates. The other group, named by Schaudinn Neosporides, includes the Microsporides, Myxosporides and Actinomyxides. It approximates more closely to the Amœbina, certain varieties being capable of amœboid movement. The Neosporides are distinguished by the formation of special reproductive cells (cnidospores), which always possess a hard bi- or tri-valved shell, several (at least four) nuclei, and one to four polar nematocysts. These consist of a vesicular wall or capsule surrounding a cavity filled with fluid, containing a long and usually spirally coiled thread continuous with the wall

of the vesicle (fig. 3). This thread is shot out with great velocity, either spontaneously or under the influence of certain stimuli. Of the several nuclei of the cnidospore, a certain number (generally two) go to the formation of the soft bodies (amœboid substance of sporoplasm) which escape when the shell opens; others (cnidoblast nuclei) play a *rôle* in the formation of the nematocysts; while others again (shell nuclei) form the shells. On account of their possession of the characteristic nematocyst or "cnidoblast," the Microsporides, Myxoporides and Actinomyxides are usually classed together under the name of Cnidosporides. The Sarcosporides are usually included in this group, in spite of the fact that they possess neither shell nor cnidoblast and have one nucleus only.

Many different methods of inducing the cnidospore to discharge the nematocyst have been recommended, but of these none is invariably successful. The best method is to examine the organism in the fresh gastric or intestinal secretion of its host. In some cases, the addition of iodine (solution, tincture, or iodine in iodide of potassium) is attended by good results; while with other species distilled water, boiling water, potassium solution, ammonia, strong mineral acids (nitric, sulphuric), ether, and other chemical reagents will achieve the desired effect. In a few cases, mechanical pressure is a sufficient stimulus, Practical experiment is, however, the only means of determining which of these measures will react upon the particular species under observation.

Order 1. *Microsporidia.*

The Microsporides are recognized by the peculiar structure of their cnidospores, which are extremely small, either pear-shaped, oval, or oblong in form, and appear always to contain four nuclei. They are furnished with never more than one large nematocyst, which, as a general rule, can be seen only after treatment with reagents. The Microsporides are very numerous and are found in the Arthropods (crustaceans, arachnoids, insects), in fish and in the tortoises. They are said to occur in man, but the assertion lacks proof. Certain species inhabit the hollow organs of their host, others are cell or tissue parasites.

From a practical point of view, the most important of the Microsporides is undoubtedly *Nosema bombycis*, Næg., the originator of the "Pebrine" of silkworms. It is peculiarly deadly to the silkworm, and penetrates into every organ of its host. Its small size, however, renders it extremely difficult of detection. When treated with concentrated nitric acid the entire organism swells and the nematocyst becomes visible; some varieties will even project their thread. Under

natural conditions, this occurs as a result of the chemical action of the intestinal secretion of the caterpillar, and immediately afterwards the naked amœboid germ emerges from the cnidospore and buries itself in the epithelial cells of the intestinal canal. The parasite now multiplies rapidly by repeated binary fission. The process of separation, however, is frequently incomplete, and the daughter-individuals thus form themselves into long chains resembling a rosary, at right angles to the free surface of the affected epithelial cell. The infection spreads with great rapidity from the intestine to all the other organs of the host, and, eight days after the ingestion of infected material, the entire body of the caterpillar swarms with parasites. Wherever, as the result of this rapid increase, there is an insufficiency of nutriment or of space, the individual parasites form cysts and become converted into cnidospores. These may be found in the intestinal tract as early as three days after infection. Not only is infection conveyed directly, by means of fæces containing parasites, but it may be congenital, the eggs of the mother having become tainted. The organism is transmissible to other varieties of caterpillar, as, for instance, *Arctia caja*.[1] Many caterpillars, both exotic and native, support parasites apparently similar to *Nosema bombycis*, but of which little is known. If affected caterpillars are obtainable, cover-glass preparations and, more important still, thin sections should be made from them. The latter should not exceed 2 μ in thickness and should be prepared from organs, preferably the intestine. They should be fixed in alcoholic solution of mercuric chloride.

The common cockroach, *Periplaneta orientalis*, harbours a parasite which is well worth studying. It is a Microsporide known as *Pleistophora periplanetæ* (Lutz and Splendore),[2] and is found in the Malpighian vessels of its host. The intestine should be removed in the manner already described, and the long, fine Malpighian vessels, which enter the intestine at the junction of the middle with the terminal portion of the intestinal tract, should be carefully separated and freed from fat. For examination they should be cut in small pieces and put into saline solution. The parasites show amœboid movement; their diameter varies from 0·002 to 0·055 mm., and the number of their nuclei from one to sixty or more. Where infection is very severe the cells of the Malpighian vessels become pathologically changed (less granulated than the normal), although the parasites

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[1] Stempell, "Die Pebrine-Krankheit der Seidenraupe," Sitzber. d. med. naturwiss, Gesellsch. Münster i. W., 1907. Meeting of June 25, 1907.

[2] W. S. Perrin, "Observations on the Structure and Life-history of *Pleistophora periplanetæ*, Lutz and Splendore," *Quarterly Journal of Microscopical Science*, vol. xlix, 1906, pp. 615-633.

occupy the lumen of the vessel only, and never take up an intracellular position. The degree of infection is augmented by the multiplication of the parasites by means of fission or budding. Infection is conveyed to fresh hosts by the formation of cnidospores. Rounded parasites, the so-called "sporonts," are found in the terminal portion of the gut as well as in the Malpighian vessels. They contain numerous cnidospores, with remains of old protoplasm attached. Single spores are found in the fæces.

When cut into small portions the Malpighian vessels may be prepared as cover-glass specimens. They should be fixed in alcoholic solution of mercuric chloride and stained with iron-hæmatoxylin. It will be found quite easy to follow the process by which a single homogeneous nucleus divides twice and produces a fourfold nucleation. The material may also be dried on cover-glasses and stained by Romanowsky's method; here the results are more brilliant but less natural. To localize the centre of infection, fix the Malpighian vessels whole, embed, and cut into sections.

The method of multiplication of *P. periplanetæ*, both by fission and by means of spores, constitutes a true alternation of generation. The phenomenon is observed in other Microsporides, as, for instance, in *Thelohania mülleri* (L. Pfr.), found in the muscular structure of *Gammarus pulex*, and *T. legeri*, Hesse, found in the fat corpuscles of the Anopheles larvæ (these forms are extremely small and each sporont contains only eight spores). It is not met with in the same well-defined form, however, in other members of the Neosporidian class.

An excellent example of a Microsporide of complex development is furnished by *Glugea anomala*, Mon., a parasite of the stickle-back. These organisms cause cysts, several millimetres in diameter, to appear under the skin, on the gills, and in the ovary. Cover-glass preparations of these cysts, whether fresh or fixed and stained with iron-hæmatoxylin, are useful only for the examination of the cnidospores. The latter are furnished with a very long nematocystic thread, which is projected when the specimen is treated with tincture of iodine and left in a damp chamber for twenty-four hours. For further study of the organism the entire cyst should be fixed in alcoholic solution of mercuric chloride and cut into sections. It will then be seen that the cell plasma contains numerous nuclei. In *Pleistophora* and *Thelohania* multiplication appears to result from the division of these nuclei, nuclear division being sometimes followed by the division of the entire cell. Within the homogeneous mass of protoplasm, and separated from it by a thin membrane and, in later stages, by a layer of fluid also, uni-nucleate protoplasmic bodies are found which must be regarded as the products of internal budding. These are usually

termed "pansporoblasts." They correspond to the sporonts of *Pleistophora* and *Thelohania*, and, after repeated division of the nucleus, they break up into a varying number of cnidospores. The cnidospores are at first uni-nucleate but, by repeated division, the nucleus becomes fourfold. Of these four nuclei, one forms the nematocyst and another the shell, while the other two form the amœboid germ, which, later, emerges from the shell. The manner in which fertilization takes place is not exactly known, but it is probable that, as in the Amœbæ, it is by autogamy. In older cysts the protoplasm is reduced to a thin wall, the whole of the interior being occupied by a hollow space filled with cnidospores.

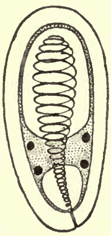

FIG. 3.—Cnidospore of *Glugea anomala*. Diagrammatic. (After Stempell.) (Magnified about 5,000 : 1.)

In place of *Glugea anomala*, an example of a Microsporide which forms large cysts is furnished by *Glugea lophii*, Dofl. This parasite inhabits the axis-cylinder processes of the ganglion cells of *Lophius piscatorius*, which is fairly common in the Mediterranean, and may be obtained from one of the zoological stations in that neighbourhood.

Order 2. *Myxosporidia.*

The Myxosporides are distinguished by certain structural peculiarities of the cnidospores. These are formed in pairs within a pansporoblast; they are enclosed in a bivalve shell; and their nematocysts—two or, more rarely, four in number—are projected in response to very slight stimulus. In the case of a few very minute varieties, found in the gall-bladder of fish, the entire organism forms a single pansporoblast which dies after the formation of the cnidospores. The large majority of the Myxosporides, however, appear as plasmic bodies of so large a size as to be almost visible to the naked eye. The interior of these bodies, like that of the microsporide, *Glugea*, is filled with numerous pansporoblasts which are definitely separated from the surrounding plasm. The Myxosporides are widely distributed among fish and are found, though with less frequency, among other cold-blooded animals. They occur either in the hollow organs (gall-bladder, urinary bladder, uriniferous tubules), where they perform amœboid movements, or they occur as tissue parasites.

(*a*) *Myxidium lieberkühni*, Bütschli.

Of those varieties of Myxosporides which inhabit the hollow organs and are capable of amœboid movement, the most readily obtainable is *Myxidium lieberkühni*. It occurs, sometimes in very large numbers,

in the urinary bladder of nearly all pike. It should be sought only in freshly killed fish, as in a dead host it soon perishes.

For the study of the living organism, the whole of the small urinary bladder, which, in the pike, lies on the dorsal side of the terminal portion of the intestinal canal, should be removed and opened and its contents examined in the fresh state. Hairs or glass threads should be introduced under the cover-glass to prevent the Myxides from being harmed by pressure. The organisms are seen to be very slow in their movements; their shape, which changes, may be said to be ribbon-like; there is marked differentiation of the endo- from the ectosarc. The hyaline ectosarc is particularly well developed in parasites which live free in the lumen of the bladder; in those which are attached to the walls of the organ it is less clearly defined. The endoplasm is surrounded by a layer of material, sharply separated from it and finely granulated. The principal mass of protoplasm is coarsely granular and rich in varied contents (spores, yellow granulations, oil globules, and hæmatoidin crystals).

The superficial layer surrounding the endoplasm may be seen with great distinctness if a drop of weak watery solution of eosin be introduced under the edge of the cover-glass. The superficial layer becomes a light rose-pink and is easily distinguished both from the uncoloured ectoplasm and the comparatively deeply stained endoplasm.

The cnidospores are long in shape with a nematocyst at either end. Two are formed within each pansporoblast, and they are always found in pairs. They vary very much in numbers. It is probable that during the winter months Myxides do not form spores at all.

Cover-glass preparations of urine containing Myxides are useful for observing cnidospores. They should be stained with iron-hæmatoxylin. The method is not practicable for the preservation of the whole organism, however, the Myxides being easily broken and crushed in manipulating the thin smear. For this purpose, sections should be made as follows: The whole of the infected bladder should be removed and emptied of urine through the artificial orifice. It should be filled with a fixing fluid by means of a pipette, and then immersed in the same fluid in a shallow dish. Alcoholic solution of mercuric chloride and Flemming's mixture are the best for this purpose. The sections show Myxides in large numbers upon the epithelial surfaces. Of these, some are furnished with a tapering front portion, by means of which they are attached to hypertrophied cells; in others, this frontal portion is broadened out into a sort of solelike attachment, by means of which they remain upon the epithelial surface.

(*b*) *Sphæromyxa labrazesi*, Lav. et Mesn.

Myxidium lieberkühni is not a very good subject for the study of cnidospore formation. Not only is the plasm rich in contents, but, unlike the other Myxosporides, sporulation is simultaneous, thus allowing one developmental stage only to be observed at a time. The parasites found in the gall-bladder are better subjects for this purpose and, of these, Schroeder[1] considers *Sphæromyxa labrazesi*, a tenant of the sea-horse, the most favourable. Fresh material is readily obtainable as, up to now, every specimen of *Hippocampus brevirostris*, Cuv., and *H. guttulatus*, Cuv., examined at Arcachon and Rovigno has been found to contain this parasite. The living organism is of little value for purposes of investigation, however, and fixed and coloured specimens are infinitely to be preferred.

Sphæromyxa labrazesi is a flat, disclike Protozoon nearly circular in shape. It may attain a diameter of 5 mm., and it never exceeds 0·025 to 0·04 mm. in thickness. In colour it is whitish, and presents so striking a contrast to the green gall that it may even be seen through the walls of the gall-bladder. Amœboid movement, especially of the larger individuals, has never been definitely proved. The endoplasm contains a large number of vacuoles. Cover-glass preparations are not very satisfactory. It is better to fix the whole gall-bladder in alcoholic solution of mercuric chloride or Flemming's mixture, cutting sections while the bladder is in the fluid, as this facilitates the penetrative action of the reagent.

The many nuclei, which are seen surrounded by a small quantity of plasm at the nodes of the alveolar network, are of two distinct types. Either they are large and incompact (female?), or they are small and compact (male?). In the small masses of plasm which lie between each mesh of the network, there are usually two nuclei, one of each sort. It is evident that this is a case of commencing autogamy, although the two nuclei do not combine until a good deal later; not until, in fact, each nucleus has divided several times.[2] These plasmic masses containing two nuclei are the pansporoblasts of the Myxosporide. In the beginning, owing to the influence of the vacuoles by which they are surrounded, they are amœboid in appearance, but in a later stage of their development they round off and acquire a firm outline. The two original nuclei continue to

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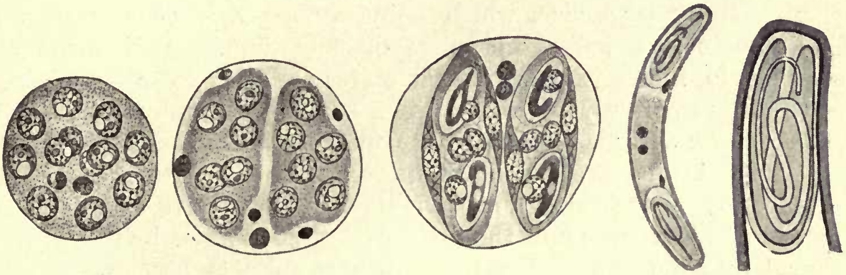
[1] O. Schroder, "Beiträge zur Entwickelungsgeschichte der Myxosporidien," *Verhdlg. d. Naturk.-med. Ver. Heidelberg, N.F.*, vol. vii, 1907, pp. 455-466; *Arch. f. Protistenkunde*, vol. ix, 1907, pp. 359-381.

[2] Compare upon this point, as also upon the analogy between Myxosporides and Amœbæ (especially *Entamœba coli*), M. Hartmann, "Das System der Protozoen," *Arch. f. Protistenkunde*, vol. x, 1907, p. 143.

divide until there are fourteen nuclei in all, eight of which are usually grouped in a peripheral circle round the other six (fig. 4, *a*). During the next stage the pansporoblast divides into halves (sporoblasts), each of which contains six nuclei, while the remaining two nuclei, which from the beginning have been noticeable for their slightly smaller size, are extruded as abortive and perish (fig. 4, *b*). The six-nucleated sporoblasts now release the cnidospores and, in this manœuvre, two nuclei, which are not visible in the ripe spore, are utilized to form the two valves of the shell. Two other nuclei play a part in the formation of the two nematocysts, which, as in the Myxides, are placed at each end of the sickle-shaped cnidospores, and to which, even in the adult spore, remains of the old nuclei may be



a *b* *c* *d* *e*

FIG. 4.—Formation of the cnidospores of *Sphæromyxa labrazesi* (after Schröder). *a*, Pansporoblast after completion of nuclear division; the old nuclei, which are to be extruded, are recognizable by their smaller size. *b*, Pansporoblast after division into two sporoblasts; rejection of the two old nuclei. *c*, Pansporoblast with young cnidospores, showing the cells and nematocysts in process of formation. *d*, Finished cnidospore. *e*, A nematocyst. (*a* to *d* magnified 1,200 : 1; *e* about 2,500 : 1.)

seen adhering. The two central nuclei form the nuclei of the amœboid germ of sporoplasm (fig. 4, *c* and *d*). These two nuclei of the sporoplasm (each of which is *probably* derived from one of two other nuclei which combined to form the pansporoblast) finally refuse to form a single nucleus.

(*c*) *Myxobolidæ.*

Small protuberances are frequently seen on the gills of fresh-water fish. They are either round or oval in shape and, owing to their white colour, they are easy of detection. They are the so-called "cysts" of Myxosporidian origin, and belong to the family Myxobolides.

The milky contents of these cysts should be examined in the fresh state. It consists almost entirely of cnidospores furnished with two nematocysts, which are placed together at what is usually termed the "front" pole. Two Myxobolides are recognized; they

are distinguished by the shape of the adult spore. They are *Myxobolus*, with round or oval cnidospores ; and *Henneguya*, the cnidospores of which are drawn out into a long, tail-like process, at the opposite end to that at which the nematocysts are placed. This process is a prolongation of the shell only, which is bivalved and surrounds the entire cnidospore in a double-contoured envelope. At the meridian, along which the two halves of the shell join, its substance is thickened into a kind of roll, which is clearly seen when the cyst is viewed in profile. Different species are recognized by slight differences in the form and size of the cnidospores. The sporoplasm occupies the hinder half of the inner space, and generally projects in a corner-shaped process between the hinder ends of the two nematocysts. In the interior of the sporoplasm is a vacuole of comparatively large size, which stains a deep red-brown with very dilute iodine, or iodine in iodide of potassium. This vacuole is peculiar to the Myxobolides, and serves to distinguish them from other Myxosporides (fig. 5, *b*). The nematocysts may be made to project their threads by one of the methods before mentioned.

The following experiment is interesting as illustrating the process of infection by natural means. Living cnidospores are enclosed in a morsel of filter paper and the little packet is lowered by means of a thread into the gullet of a living fish of suitable species. A glass pipe should first be introduced and the package lowered through that to avoid injury by the pharyngeal teeth. To prevent the loss of the package, a needle is fastened to the other end of the thread and fixed in the muscles of the back with the point running from tail to head. After the packet has been exposed for twenty-four hours to the action of the secretion of the small intestine, it is withdrawn, the same precautions being employed as before. It will then be found that nearly all the cnidospores have opened, releasing the amœboid sporoplasm, while all the nematocysts have projected their threads. The means by which the young cnidospores leave the intestine of their host is not known, but it is probably by way of the blood-stream. This would account for the frequency with which the gills become infected, the parasites being caught in the capillary vessels of those organs.

The nuclear conditions are best studied by fixing the cyst contents upon cover-glasses and staining them with dilute hæmatoxylin or iron-hæmatoxylin. If iron-hæmatoxylin is used the specimens should be left for a considerable time in both the mordant and the stain, on account of the extreme impermeability of the shells. But even then it will be found that only a certain number of the cnidospores take the stain, the rest remaining uncoloured. Where the method is successful, however, it is possible to distinguish the two sporoplasm

nuclei and, occasionally, also the homogeneous reproductive body into which they fuse. The nematocysts also become visible with the remains of the two nematocystic nuclei still clinging to them. A rough idea of the general structure of the cnidospores and nematocysts may be gained by fixing whole Myxosporides and clearing them in glycerine. When required for use, they may be teased out in the same fluid and embedded in glycerine jelly. As in the case of Nematodes and the eggs of Helminthes, Myxosporides should be fixed in 70 per cent. hot alcohol to which 5 per cent. glycerine is added, the alcohol being allowed slowly to evaporate.

The best method of examining the whole organism is by means of sections cut from the infected gill lamella, which should be fixed whole in alcoholic solution of mercuric chloride. It will then become apparent that the parasite is surrounded by an envelope derived from the tissues of its host. The ectoplasm is somewhat thickened, but a true cyst, forming an integral part of the parasite itself, has not been observed. The interior of the parasite is entirely filled with cnidospores in all stages of development, which are separated from one another by scanty remnants of endoplasm. The development of the cnidospores is similar to that of *Sphæromyxa*, but it is more difficult of observation. Certain forms, however, may be recognized with comparative ease. These are: single nuclei, round which a portion of plasma has collected and which were formerly believed to be pansporoblasts; the developmental stages of pansporoblasts containing several nuclei; and the final stages of spore formation.

It is now very generally recognized that the spores are not formed, in the first instance, from a plasmic body containing a single nucleus. It is probable that, in the initial stage, two nuclei, round which a portion of plasm has collected, come together to form the pansporoblast, without fusion of either nucleus or plasm (Mercier, Keysselitz[1]). Thus the pansporoblast is originally two-celled, and nuclear division does not cease (as was formerly believed to be the case) when eight to ten daughter-nuclei are formed, but continues until these are fourteen (characteristic of the order) in number, their further development resembling that of *Sphæromyxa* (fig. 5).

Myxobolides do not only occur upon the gills; all the organs, with exception of the skeleton, may be affected. The following species have a practical importance:—

(1) *Henneguya oviperda* (Cohn).—Parasitic in the eggs of the pike, which, when affected, appear white in colour. They are very

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(1) See Braun, "Parasitenlehrbuch," 4th ed., p. 135; also G. Keysselitz, "Die Entwickelung von *Myxobolus pfeifferi*, Th.," part i, *Arch. f. Protistenkunde*, vol. ii, 1908, pp. 252-275.

convenient for demonstration purposes and especially for section-cutting, the cnidospores and especially the pansporoblasts, being separated from one another by a larger quantity of endoplasm than is the case with the parasite found in the gills.

(2) *Myxobolus cyprini*, Dofl.—Parasitic in the kidney of the carp and was considered by Hofer to be the originator of small-pox, a view which has since proved erroneous.

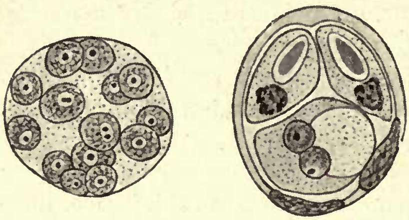

FIG. 5.—Formation of cnidospores of *Myxobolus pfeifferi* (after Keysselitz). Magnified about 2,500 : 1. *a*, Pansporoblast with 14 nuclei, at the end of nuclear division. *b*, Young cnidospore, not yet fully developed, with 2 sporoplasmic nuclei, 2 nematocystic nuclei, and 2 shell nuclei. A large vacuole is seen in the sporoplasm.

(3) *M. pfeifferi*, Thél.—Parasitic in the muscles of the barbel. It gives rise to large tumours, provoking severe pathological symptoms, which frequently end in death.

(4) *Lentospora cerebralis* (Hofer). — Resembling *Myxobolus* but differentiated by the vacuole, which does not stain with iodine. Parasitic in the cartilaginous portions of the skull of certain varieties of *Gadus*. Has been transmitted to young Salmonidæ in fisheries by feeding them with infected fish-meal, when it gave rise to a disease, the symptoms of which resemble gid and which is known in Germany as "Drehkrankheit." Both the affected cartilage (skull, in the region of the centre of hearing) and the skeleton in its immediate vicinity (vertebræ of the neck, gill-cover, gill-arches) became destroyed by granuloma.

Order 3. *Actinomyxidia.*

The Actinomyxides are interesting on account of the unusual formation of their cnidospores. The five species known to us are parasitic in certain varieties of the Oligochætes family of the Tubificidæ ; four of them being found in the intestinal epithelium of freshwater worms, while the fifth inhabits the body-hollow of a marine worm. The cnidospores vary with the species, some being round, others furnished with processes of different kinds, which may give the whole cnidospore the form of an anchor. They are, however, always eight in number and are arranged like a three-rayed star; they are furnished with a trivalve shell; and they have three nematocysts arranged together at one pole.

Fresh material should be carefully teased out. Cover-glass specimens and sections should be finished damp, the former to be prepared from teased-out portions of organs, the latter to be cut in series through the entire body of the infected worm.

Order 4. *Sarcosporidia.*

Sarcosporides is the name given to certain parasites, either tubular, spindle-shaped, or oval in form, which occur in the muscular structure of mammals, birds and reptiles. The great majority of these are found in the striated muscle-fibres and nowhere else ; but there are a certain number of varieties of which the early stages only are found in the striated muscle-fibres. These they destroy in the course of their development and, surrounded only by the sarcolemma, they are apparently retained in the interstitial connective tissue. A single species, parasitic in the kangaroo, is an exception to this rule, being found in the submucosa of the intestine (first stage of a parasite of the unstriated muscles ?).

The Sarcosporides, like many of the Cnidosporides, form numerous spores by the agency of a brood-cell (pansporoblast). The life of the parent organism does not cease with the completion of the process of reproduction ; on the contrary, the parent organism continues to grow and the number of spores which it produces to increase. Unlike the Cnidosporides, the spores of the Sarcosporides are not enclosed in shells. They are bean- or sickel-shaped ; they have one nucleus ; and they are not furnished with a nematocyst. The whole organism is surrounded by a membrane in which, as a rule, two distinct layers are recognized, the inner layer being thin and hyaline. The outer layer, which is not always very distinct but can generally be seen in the later stages, is furnished with a striation running at right-angles to the surface. This has been variously interpreted ; it may be due to the presence either of rodlike structures or of pore-canals. The latter alternative seems the more likely.

Little is definitely known of the life-history of the Sarcosporides. The species parasitic in the swine and the sheep are readily obtainable, and these should be examined.

Sarcocystis miescheriana (Kühn), the Sarcosporide of swine, is frequently discovered when examining the carcass for Trichinæ. It is found in the midriff, the intercostal muscles, and the muscles of the larynx (according to Kühn, in 98 per cent. of swine). Ostertag has frequently found it in the abdominal muscles, and it may occur in any of the striated muscles, including the heart. In this it differs from Trichinæ, which are never found in the muscular structure of the heart.

The organism should first be examined in the fresh state. A small portion of the infected muscle is cut out with a fine pair of scissors and examined in normal saline with a low-power lens, the cover-glass being gently pressed down on to the specimen. Within single muscle-fibres, rod- or spindle-shaped forms, rounded off at each end and measuring ¾ to 1 mm. in length by 0·08 to 0·01 mm. in thickness,

will be seen. Being comparatively opaque, they strike the eye at once by the unrelieved greyness of their colour and by their close granulation. If the morsel of flesh is teased out in normal saline or, better still, in lymph, and a muscle-fibre containing a single Sarcosporide is examined with a high-power lens, it will be seen that the interior of the organism is filled with minute spores. By teasing out the Sarcosporide itself, the individual spores may be isolated and examined, though the method is successful with the larger varieties only (see later, *Sarcocystis tenella*).

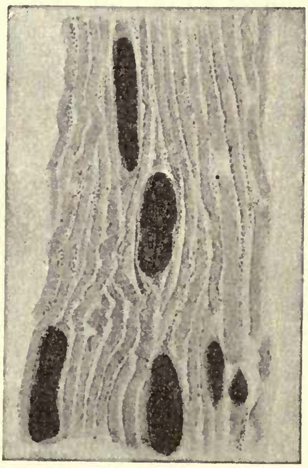

FIG. 6. — Longitudinal section through a muscle from the pig, containing *Sarcocystis miescheriana* (Kühn). Magnified 30 : 1. (After Braun.)

Coloured preparations of whole Sarcosporides are made as follows: Small portions of infected flesh should be fixed with picric acid (saturated watery solution of picric acid 50 parts, aqua destillata 48 parts, glacial acetic acid 2 parts; time, according to the size of the specimens, half to two hours; in any case, until they become quite yellow). The specimens are then put into 50 per cent. alcohol for a short time (about fifteen minutes); and afterwards into 70 per cent. alcohol, which must be repeatedly changed; and finally, into 80 to 90 per cent. alcohol. They should be stained whole with borax-carmine (twelve to twenty-four hours) and afterwards differentiated in alcoholic solution of hydrochloric acid. After clearing in cedar-wood oil or creosote, the muscle is teased out in the clearing fluid and the infected muscle-fibres separated. The brilliant red colouring which the Sarcosporides take on is in such sharp contrast to the pale muscle-fibres, that the former may be recognized with the naked eye.

Sections should be prepared as follows: Fix in alcoholic solution of mercuric chloride by Schaudinn's method, or in Rath's picrin mixture. Colour the sections with hæmatoxylin and eosin. Cross-sections are particularly interesting; they show more distinctly than either longitudinal sections or whole preparations the way in which the muscle-fibres containing the parasites become thickened.

The Sarcosporide of the sheep, *S. tenella*, Raill., is likewise found during its earlier stages in the interior of the striated muscle-fibres. It was found by Bertram at Rostock in 182 out of 185 sheep. It grows to a much larger size, however, in the muscles of the larynx and pharynx (Ostertag says also in the skin and abdominal muscles)

of the sheep than it does in those of the swine. It forms oval knots several millimetres in diameter which, under certain conditions, may attain the size of a hazel-nut. This stage in the development of the parasite was formerly known as *Balbiania gigantea.* Cross-sections of these knots show a very distinct alveolar network. Branching partitions, formed of an anastomosing membrane, run from the surface to the interior of the organism, enclosing numberless irregular polyhedral chambers which do not communicate with one another. This alveolar meshwork is also present in the Sarcosporide of swine, but, as the chambers and spores are densely crowded, it can be seen only in very thin sections or after breaking and emptying the individual chambers. Each chamber represents a pansporoblast which has divided into numerous spores. In the large Sarcosporides of sheep, spores are found in those chambers only which lie near the surface of the organism ; in the interior chambers they are broken up into a granular mass.

In order to examine single spores, one of the large Sarcosporides from the gullet of the sheep should be teased out in natural lymph or in normal saline. The naked spores are seen to be bean- to crescent-shaped. Their plasm is finely granular and at one pole assumes a differentiated structure, becoming, for about one-third its length, somewhat paler in colour and obliquely striated. This modification has been compared, though upon insufficient grounds, with the nematocyst of the Myxosporides. Active movement in the spores of the Sarcosporides of the swine and the sheep has not been proved. It has, however, been observed in the Sarcosporide of the house-mouse, where it takes the form of a forward progression, starting in jerks by means of a screwing movement round the long axis of the organism. For demonstration purposes, the parasites should be taken from a freshly killed mouse and examined in a micro-incubator at blood temperature. Osmotic changes and mechanical movements may easily be mistaken for spontaneous movements.

The spores should be stained in the following manner: A cover-glass preparation of a single Sarcosporide is fixed in alcoholic solution of mercuric chloride. It is coloured with hæmatoxylin or iron-hæmatoxylin. The pole opposite to that at which the nucleus is situated will appear paler and free from granulation, but a thread-cell or nematocyst, such as is seen in the Myxosporides, is not present. The single nucleus is comparatively large in size ; it is oval in shape, being slightly elongated in the axis of the spore, and it is placed very close to one polar extremity.

Sarcosporides have been found in other mammals besides the sheep and swine, as well as in several saurians (*Platydactylus mauritanicus, Lacerta muralis*), and a large number of birds. In the few

authentic cases of human infection by Sarcosporides (one case in Egypt, that of a Sudanese, two cases in France), the particular parasite would appear to have been *Sarcocystis tenella.* Otherwise, it is customary to distinguish the species of Sarcosporides by giving them the names of their hosts. In addition to the varieties already mentioned, the following have a practical interest :—

(1) *S. bertrami,* Dofl.—Found in the muscles of the horse, especially in those of the throat and neck. Siedamgrotzky found it in all the horses which he examined for it in Dresden. Many veterinary authorities believe that the so-called "ice-ball swellings" (Eisballengeschwülste) of horses are due to the presence of this variety of Sarcosporide, the young spores migrating into the neighbouring tissues after the bursting of the sac. There is, however, no definite grounds for this belief, as such a developmental stage does not approximate to any detail in the life-history of other and better known Sarcosporides.

(2) *S. blanchardi,* Dofl.—Found in the muscles of the ox and the Java buffalo. According to Sanfelice, it is present in the tongue of nearly all Sicilian cattle.

(3) *S. muris* (R. Bl.).—Found in the rump muscles of the house-mouse and the common rat. Remarkable for the length (several centimetres) which individuals sometimes attain. It differs from *S. tenella* in that it does not completely destroy the muscle-fibre in which it occurs. The parasite has, however, a special significance; Koch and Smith found it possible to bring about an artificial infection of mice by feeding them with Sarcosporides. Negri[1] believes that he has observed reproduction of *S. muris,* as of *S. bertrami,* by simple binary fission lengthwise of the spore.

Class III. **Flagellata.**

The Flagellates, or whip-cells, are distinguished by the possession of one or more flagella. These organisms are particularly important as being the originators of a number of very dangerous diseases. Unlike the Amœbæ, little is to be gained by the examination of free-living forms, the difficulty of obtaining material outweighing any advantage to be derived from their study.

Before entering into a description of the various species of Flagellates, it is advisable to consider the characteristics of their flagellate apparatus. This may assume various forms, the most important being as follows :—

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[1] A. Negri, "Beobachtungen ueber Sarcosporidien," *Centralbl. f. Bakt.*, &c., part i, No. 47, 1908, pp. 56-61.

(1) *Principal flagella,* which spring from the anterior end of the organism and are projected forwards. They occur singly or in numbers.

(2) *Secondary Flagella.*—Smaller processes, placed near the true flagella, and likewise occurring singly or in numbers.

(3) *Trailing Flagella.*—Comparatively long processes which always occur singly. The trailing flagellum starts at the anterior end, generally behind the true flagellum, and it trails behind as the organism swims through the water. These flagella occasionally assume an active part in the phenomena of movement, when they perform jerking movements and so act as a rudder. Some Flagellates are able to bring themselves to an anchor by means of their trailing flagellum.

(4) *Undulating Membranes.*—This process is found solely among the parasitic members of the group, and it always occurs singly. It is formed by a flagellum, which is attached to the parasite along its length by means of a delicate lamella of protoplasm. The flagellum appears as a thickened edge upon the undulating membrane, and may be continued as a whiplike process beyond it. This is not, however, invariably the case, and the undulating membrane itself is subject to considerable modification.

The flagellum, whatever its form, is always connected with the nucleus by a characteristic basal structure. At the spot where the flagellum takes its rise, a granule (blepharoblast) is present in the outer layer, which communicates as a rule with the nucleus by means of a strong fibrillum (rhizoblast). In many Flagellates, classed together by Hartmann under the name "Binucleata," there is, in addition to the principal nucleus, a special locomotor nucleus called variously "flagellar-nucleus," "blepharoblast," or "kineto-nucleus."

In the life-history of almost all Flagellates, definite periods occur during which there is a temporary degeneration of the flagellate apparatus. This period frequently coincides with the stages of fertilization and encysting. The phenomenon has a special significance in the case of certain parasites (particularly the intestinal parasites of insects and the blood parasites of vertebrates) which are able, in the absence of the flagellum, to attach themselves to the cells of their host or even to penetrate to the cell interior.

Flagellate organisms obtain their nourishment in a variety of ways. The parasitic varieties feed either by endosmosis (*Bodo, Trypanosoma*) or by the ingestion of solids (*Trichomonas, Trichomastix*). Those Flagellates which ingest solid food are furnished with a special oral part, the cytostome (fig. 7, *b* and *c*), situated at the anterior end of the body. The methods of nutrition have a practical importance in the cultivation of Flagellates upon artificial media.

Classification is based mainly upon the structural peculiarities of the flagella. The subclass Euflagellata is the only group which includes parasitic forms, and of this subclass we shall concern ourselves with the orders Protomonadina, Polymastigina, and Binucleata.

Order 1. *Protomonadina.*

The Protomonadines, which must be regarded as the most primitive of the Flagellates, are capable of more or less definite changes of body-form, some varieties performing characteristic amœboid movements. These organisms are extremely minute; they have a single nucleus; they do not possess an undulating membrane; and they are furnished with two flagella, placed at the anterior end. The parasitic varieties are the Cercomonades and the Bodonides, the former possessing one flagellum, the latter being furnished with two, one principal and one a trailing flagellum. Very little, however, is known about these varieties, and this applies particularly to those forms observed in human excreta. The best known is *Bodo lacertæ* (Grassi),[1] which occurs with great frequency in the cloaca and in the terminal portion of the intestinal canal of lizards. Being readily obtainable, it is useful for demonstration purposes, illustrating as it does the simplest form of flagellate apparatus.

A small quantity of fæces is expelled by gentle pressure from the anus of the lizard and caught in a clean watch-glass. If the material is to be examined fresh, the fæces should be diluted with a little normal saline. To reduce the motility of the parasites, add a small quantity of carragheen which has been previously soaked in normal saline.

In addition to *B. lacertæ* and the Amœbæ (already described), two varieties of Polymastigina are found in preparations of this kind. These are easily recognizable by the large number of their flagella and their more compact appearance (fig. 7).

B. lacertæ (fig. 7, *a*) appears at a first glance to be pear-shaped, but, as a matter of fact, is flattened and rolled inwards in a spiral fashion, so that the parasite resembles a coarse gimlet. Two flagella of equal thickness spring from the anterior end, one of which is usually projected forward, while the other and shorter of the two trails behind. There is no cytostome, and it is assumed that nutrition takes place by endosmosis.

The more intimate structural details are best seen in finished cover-glass specimens. These should be fixed in alcoholic solution of

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[1] S. Prowazek, "Untersuchungen ueber einige parasitische Flagellaten," *Arb. a. d. Kaiserl. Gesundh.-Amte*, vol. xxi, 1904, pp. 1-41.

mercuric chloride, and coloured with iron-hæmatoxylin. They show distinctly the two granules which lie close together at the base of the two flagella, as well as the fibrillum or rhizoblast which runs from each granule sideways to the nucleus.

B. lacertæ undergoes reproduction in several ways. Round brood-cysts are sometimes seen, which are formed by the secretion of a soft, jelly-like membrane after complete degeneration of the flagellate apparatus, and within which two to four daughter-individuals are formed. During other stages of its development, the parasite multiplies, without encysting, by longitudinal fission, in the course of which an equatorial plate is formed, the nuclear mechanism evidently aiming

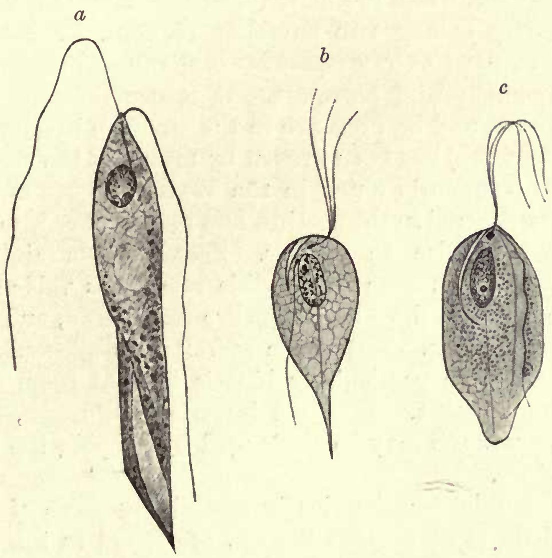

Fig. 7.—Species of Flagellates which are parasitic in the cloaca of lizards. (After Prowazek, slightly modified.) *a*, *Bodo lacertæ* ; *b*, *Trichomastix lacertæ* ; *c*, *Trichomonas lacertæ*.

at an exact division of the chromatin. Permanent cysts are also formed, by means of which infection is conveyed. These cysts are so highly refractive that little is to be learnt from them in the living state. Cover-glass preparations, however, when stained with iron-hæmatoxylin, show that autogamous reproduction takes place within them.

Order 2. *Polymastigina.*

The Polymastigina are Flagellates with one or two nuclei and with four to eight flagella, the latter differing more or less in structure. In the binucleate varieties, the nuclei are similar in form and size. To this class belong the parasites *Trichomonas* and *Lamblia*, which differ

from one another considerably in structure, however. Quite distinct from these, again, is *Costia*, a parasite met with among fish.

(*a*) *Trichomonas and Trichomastix.*

The Trichomonas are characterized by their small size, their pear-like shape, and by the possession of four flagella which, with the cytostome, are placed close together at one end. Of these, three of about equal length are true flagella, which frequently adhere to one another at the base, while the fourth forms an undulating membrane.

To this group belong two parasites of man, *T. intestinalis* and *T. vaginalis*. *T. intestinalis* is a parasite of the small intestine, frequently met with but apparently harmless. It is occasionally found in the stools in diarrhœa, and its presence in the stomach in gastric affections (especially carcinoma) has been proved by means of the stomach-pump, *T. vaginalis* is frequently found in the vaginal mucus of women. It may also be transferred to the urethra and pass thence into the bladder, where it may give rise to cystitis. Secretion containing parasites should be examined as soon as possible after it is voided. Owing to their extreme activity, the Flagellates are easily seen, and especially so in centrifugalized urine. But if the secretion is allowed to stand for some time, and more particularly if it is kept at room temperature, active movement ceases. Like the intestinal Infusoria, however, the activity of *T. vaginalis* may be prolonged by keeping the secretions at body temperature.

Varieties of Trichomonas closely resembling those of man, and of which very little is at present known, are found in the intestines of different mammals, as also in the guinea-pig, mouse, and rat. These are very useful for demonstration purposes. A similar variety, *T. lacertæ*, Prow. (*nec* Dofl.), is found in the rectum of Lacertians; it is, however, somewhat rare. Another form closely resembling it, *Trichomastix lacertæ*, Blochm., is more frequently met with in the rectum of lizards (both *Lacerta agilis* and *L. muris*), and this will well repay examination. It differs from Trichomonas in having no undulating membrane, the flagellum, which is turned backwards, forming a free trailer. Moreover, the three principal flagella proceed from a basal structure placed upon one side of the organism, while the trailing flagellum proceeds from another similar structure upon the other side, and quite distinct from the first. In Trichomonas, on the other hand, all four flagella seem to emanate from one large four-cornered basal structure (fig. 7, *b* and *c*). These varieties are obtained and examined in the manner described on p. 38.

(*b*) *Lamblia.*

The third variety of Polymastigina found in man, *Lamblia intestinalis* (Lambl) is, like *Trichomonas intestinalis*, R. Lckt., an inhabitant of the small intestine, where it attaches itself to the epithelium by means of a sucker-like depression in its broad anterior end. In addition to this characteristic sucker-like depression, the Lamblia are distinguished by their symmetrical bilateral structure; by the peculiar tapering form assumed by the motile posterior part; by the possession of four pairs of flagella, of which one pair is placed at the front edge and two pairs at the hinder edge of the sucker-like depression, while the fourth pair proceeds from the posterior tip of the body; and further, by the possession of two similar and symmetrically situated nuclei, which together have something the appearance of a dumb-bell. Each flagellum is provided with a characteristic basal structure, from which a fibrillum runs to one of the two nuclei. By careful examination, this fibrillum may be seen as a fine dark line in the living organism; in specimens stained with iron-hæmatoxylin it takes on a still deeper colour.

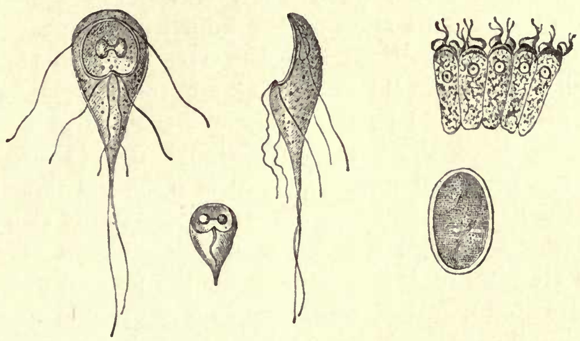

FIG. 8.—*Lamblia intestinalis:* full view; profile view; dead upon the intestinal epithelium; encysted. (After Grassi and Schewiakoff, from Braun.)

Forms similar to that parasitic in man and considered by many to be identical with it, are found in the intestine of mice and rabbits. These are useful for purposes of investigation. It should be borne in mind that the parasites are rarely present in the bowel contents; they must be removed from the intestinal epithelium to which they will be found adhering.

(*c*) *Costia.*

There is a third species of Polymastigina of which a passing mention must be made. This is *Costia necatrix* (Henn.). It is ecto-parasitic upon the skin and gills of freshwater fish and may acquire

a pathological significance in hatcheries and aquaria. Four flagella spring from that end of the organism which is posterior when swimming, and of these the two longer serve to attach the parasite to the skin of its host, while the shorter pair are employed to obtain food. These parasites excite an increased secretion of mucus on the part of their host, which causes a characteristic dulness of the skin in fish so affected. Owing to this increase in the mucous secretion, the parasites may be damp-fixed in cover-glass preparations.

Order 3. *Binucleata.*

(*Hæmoflagellates and Hæmosporides.*)

These Protozoa are parasitic in the blood of vertebrates. Except in the case of certain species (Trypanosomes), their flagellate nature is not immediately apparent. In the greater number of species, the adaptation of the organism to cell-parasitism has so completely modified the flagellate apparatus, that it is customary to class them together as a special order (Hæmosporides) of the Sporozoa. Quite recently, transition forms have been discovered which render it impossible in the present state of our knowledge to draw a sharp line between the Hæmoflagellates and the Hæmosporides.[1] Hartmann includes both in the term "Binucleata" and treats them as an order of Flagellates, distinguished by the peculiar structure of their flagellate apparatus. In both there is a functional double nucleation; in addition to the principal nucleus, there is a special flagellar nucleus, with which the flagella, generally one and more rarely two in number, are connected. Although this basis of classification is not, as yet, accepted by all authors, it is expedient for the present purpose to class the blood parasites together, especially as the method of examination is the same in all cases.

(*a*) GENERAL DIRECTIONS FOR OBTAINING AND EXAMINING MATERIAL.

(1) METHODS OF OBTAINING MATERIAL.

Those blood parasites which are pathogenic in man and animals are restricted in their distribution almost exclusively to hot climates, hence fresh material is to be obtained occasionally only and by importation. In Germany, two species of blood parasite have, up to the present, been observed. These are, *Plasmodium vivax*, the parasite of tertian fever in man, which I have myself observed in the flat country

[1] See M. Lühe, "Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten," *Mense's Handb. d. Tropenkrankh.*, vol. iii, Leipzig, 1906, pp. 69-268.

around Memel; and a variety of *Babesia*, which causes the so-called "bloody urine" in cattle turned out to grass. These varieties are sporadic in their occurrence, and little reliance can be placed upon them for practical purposes. Animals bred in laboratories and stables are free from blood parasites, hence fresh material is only to be obtained from free-living animals or by importation. There are, however, many native wild animals, especially birds, as well as certain foreign animals (lizards and tortoises), which are to be bought quite cheaply from live-stock dealers, which harbour blood parasites in great variety. The examination of these is much to be recommended, not only as an introduction to the study of blood parasites in general, but as a means of increasing our knowledge in this important, but little understood, branch of parasitology.

Birds offer excellent material for examination, especially singing birds (Fringillidæ) and birds of prey (owls and falcons). Infection among them is comparatively frequent, and they are easily held and handled in the laboratory. The life-history of a Plasmodium (a member, that is, of the group to which the malaria parasite belongs) can only be followed in this country by watching the development of the *Proteosoma* of birds. The species *Hæmoproteus* and *Leucocytozoon*, though theoretically important, are confined exclusively to birds. But the very prevalence of blood parasites among birds increases the difficulty of investigation, cases of mixed infection being exceedingly common.

Free-moving Flagellates (Trypanosomes) are not found with any frequency in the blood of birds. But *Hæmoproteus*, *Leucocytozoon*, and, though in a less degree, *Proteosoma*, are by no means rare. *Hæmoproteus* and *Proteosoma* occur in the Fringillidæ (chaffinch, sparrow, goldfinch, yellow-hammer, and others), while the birds of prey (falcons, harfang and other owls) harbour *Hæmoproteus* and *Leucocytozoon*. Acute infection is seen in young birds. Of exotic bird species, that most readily obtainable is the rice-bird (*Padda oryzivora*), which I have almost invariably found to contain *Hæmoproteus*, and my experiments are confirmed by those of Laveran.

Other animals from which material may be obtained are the following:—

(1) Among *mammals*, the rat and the hamster harbour Trypanosomes. Rats bred in the laboratory do not contain parasites, but infection is by no means rare in freshly caught grey rats. The parasites once obtained, they may be permanently propagated by the repeated inoculation of white rats (see p. 45). A similar parasite is found in the hamster; it was present in every animal examined by L. Pfeiffer in the neighbourhood of Weimar, but was found by Wasielewski in only two out of twenty-eight examples caught in the

neighbourhood of Halle. This species of Trypanosome can only be cultivated in the hamster. The blood parasites of the bat are very interesting, but their study is rendered difficult by the fact that bats do not live long in captivity.

(2) Among *reptiles*, the most important are the lizard, *Lacerta muralis*, and the tortoises, all of which harbour Hæmogregarines. The wall-lizard is frequently the host of *Caryolysus lacertarum*, while varieties of Hæmogregarines are always present in the tortoise.

(3) Of *amphibians*, frogs are the most interesting, Trypanosomes and Hæmogregarines (*Lankesterella*) being frequently found in them.

(4) Among *fish*, the Cyprinides are the most important. I have rarely examined Crucians or tench without finding Trypanosomes. When mammalian Trypanosomes are not available, these fish, though they are as a rule only slightly infected, provide useful material for demonstration purposes.

(2) Inoculation.

In studying the blood parasites, it is essential to examine and re-examine fresh material at different times, for in this way only can the developmental stages of the different species be followed. A sufficiency of material is necessary to investigation ; to obtain this, the parasites (already discovered or obtained from other laboratories) should be propagated by the inoculation of living animals. The resulting infection is generally acute in character, and the animals may succumb after a definite and, generally, very short time. This is the case with mice infected with the Trypanosomes of tsetse-fly disease, and with dogs acutely infected with *Babesia*. On the other hand, the animal experimented upon may overcome the acute stage of the disease, but continue to harbour parasites in very small numbers. This is the case with singing birds infected with *Proteosoma*. In cases of mixed infection, it is occasionally possible to isolate one variety of parasite by inoculation. Among the parasites of birds, for instance, it is extremely easy to convey *Proteosoma* by inoculation, while *Hæmoproteus* is not so readily conveyed in this manner. Hence, by inoculation from a case of mixed infection, the former parasite may be obtained. The inoculation method has, however, proved its greatest value in the preservation, for purposes of demonstration and research, of numerous blood parasites which are pathogenic in hot countries. Thus, *Trypanosoma brucei*, the originator of tsetse-fly disease, is now cultivated in the laboratories of all countries owning African colonies.

It is a point of some importance that certain parasites are con-

fined to certain hosts, while others may be transmitted to animals of widely different species. Thus, *Babesia canis* will live and develop only in the dog, *Trypanosoma lewisi* only in the rat, and *T. criceti* only in the hamster; but the greater number of pathogenic Trypanosomes are transmissible to all kinds of laboratory subjects. The study of specific parasites is facilitated by choosing animals for experiment which, under normal conditions, are never infected with the organism under investigation. This applies to all the species already mentioned (indeed, that pathogenic to man can only be studied by inoculating animals), and also to the Trypanosome of rats, which may be transmitted to white rats. It applies, moreover, to *Proteosoma,* which may be transmitted to canaries, a point of considerable practical importance to the student.

The inoculation method is very valuable for observing the Trypanosomes of mammals, the *Proteosoma* and the *Babesia.*

(1) Inoculation with the Trypanosomes of rats should be performed as follows: Blood is drawn from the artery of a chloroformed rat by means of a Pravaz's syringe. It is diluted with sterile normal saline, and a quantity of 3 to 5 c.cm. is then injected, either subcutaneously or intraperitoneally, into the body of a white rat. The period necessary for incubation, that is, until the appearance of Trypanosomes in the blood, is generally about twenty-four hours, though it may occasionally be as long as four days. The period of infection lasts from three weeks to six months. There are, as a rule, no pathological appearances.

Rats may be used for the cultivation of the tsetse Trypanosome. They die very quickly, however (in six to nine days), and this necessitates frequent re-inoculation, if living Trypanosomes are to be kept for any length of time. The incubation period is up to two days. Mice are more convenient for this purpose, but they die in from three to six days.

(2) *Proteosoma* should be conveyed by the following method: A small quantity of blood is withdrawn from the wing vein of the infected bird, or, if the bird is freshly or quite recently killed, the blood may be taken from an artery. It is diluted to six times its bulk with sterile normal saline, and of this mixture 0·2 to 0·3 c.cm. is injected into the breast-muscle of a canary. The parasites generally appear at the end of about a week, and by the third week they are very numerous.

When it is desired to bring about a strong infection for demonstration purposes, and the subjects for examination are canaries which have already been infected, but in which the acute stage is passed, parasites having become so scarce in the blood that they are no longer demonstrable, v. Wasielewski recommends that two birds

should be inoculated four weeks before it is proposed to examine them. Sometimes, though not always, the resulting infection may be only slight, and for this reason the blood should be tested daily between the tenth and twentieth days. If the parasites begin to decrease in numbers, two fresh canaries should be inoculated with the blood of the first two. By this method *Proteosoma* may always be obtained in large numbers.

Of the Babesia, *Babesia canis* is the most favourable for examination purposes. Inoculation should be subcutaneous and a large quantity (15 to 20 c.cm.) of material should be used.

(3) Cultivation Outside the Body.

A certain number of blood parasites may be cultivated upon sterile media outside the body of their host. By taking suitable precautions they may not only be kept alive, but they may be induced to develop and to multiply.

Simple preservation is possible in the case of *Babesia* and of Trypanosomes, if the blood which contains them is prevented from clotting by defibrination or by the addition of soda citrate. Laveran and Mesnil recommend a solution of 5 grains of sodium chloride, and 5 grains of sodium citrate, in 1 litre distilled water, mixed in equal quantities with the blood. The duration of life varies with the species; it is longest in the case of the Trypanosomes parasitic in cold-blooded animals (fish) and in rats; it is appreciably shorter in Trypanosomes pathogenic to mammals. It depends, morever, upon temperature, and, in the case of the Trypanosomes, unlike the intestinal Infusoria, it is prolonged by cooling. For instance, *Trypanosoma lewisi* will live in summer for four days only at room temperature, while it may be kept for two months at a temperature of 5° to 7° C. Similarly, *Babesia bigemina* and *B. bovis* will retain their vitality for two months, and *B. canis* for twenty-five to thirty-nine days, if kept in a refrigerator. But in all these instances, the organism is subject to certain changes which lead eventually to decomposition. One of the most remarkable of these changes is seen in *B. canis* when examined in blood taken, under chloroform and shortly before death, from a strongly infected dog. The blood should be thinned with about an equal part of normal saline and kept at 27° C. or at room temperature. After about eighteen hours, the sediment will be found to contain parasites which, by the projection of raylike processes, have assumed a starlike appearance. They give the impression of being quite rigid, but prolonged observation in drop cultures will reveal slow changes of form. The meaning of this starlike appearance is not known, the

most probable theory being that it is a manifestation of degeneration. Koch,[1] however, observed similar phenomena in the stomach of ticks which had been previously infected with *B. bigemina*.

Citrate of soda cultures have a special interest in connection with *Leishmania donovani*, the parasite of kala-azar. By the addition of an equal quantity of 2·5 to 5 per cent. citrate of soda solution to the infected blood, the organisms not only remain alive for about four weeks, but they reproduce themselves and pass into a developmental stage which has, up to now, never been observed in man. At this stage the organism is long and narrow in shape; it possesses a flagellum, and it is actively motile. In blood taken at the height of infection, shortly before natural death, from an unchloroformed dog, active flagellate forms of *B. canis* were found. The flagella of these organisms are, however, so easily detached, that it is rarely possible to observe them in fixed and coloured specimens. It has been suggested that these flagellate forms of *Babesia* are the male sexual forms, but the conclusion is very doubtful.

Permanent pure cultures outside the body of the host have succeeded in the case of certain blood parasites, but upon two media only. These are as follows:—

(1) Blood-agar (Novy) is used for the cultivation of Trypanosomes and will be described later. It is equally useful for certain blood parasites which have become adapted to cell-parasitism. These are *Hæmoproteus* (Hartmann)[2] and *Leishmania* (Nicolle).[3]

(2) *Blood-bouillon* (Miyajima[4]) yielded remarkable results with a *Babesia* found in cattle in Japan and Corea. The infected blood was withdrawn from the jugular vein and immediately defibrinized under antiseptic precautions. It was then mixed with ordinary nutrient bouillon in a proportion of 1:5 to 1:10 and kept in sterilized test-tubes at a temperature of 20° to 30° C. Large round parasites, with a diameter greatly in excess of that of the blood corpuscles, developed in the fluid. (These appear to resemble forms found by Koch in East Africa in the stomach of ticks which had been removed two to three days previously, gorged with blood, from an ox infected with *B. bigemina*). Flagellate forms are observed in old cultures after seventy-two hours; these increase in number and are most plentiful

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[1] R. Koch, "Beiträge zur Entwickelungsgeschichte der Piroplasmen," *Zeitschr. f. Hygiene*, vol. liv, 1906, pp. 1-10.

F. K. Kleine, "Kultivierungsversuch der Hundepiroplasmen," *ibid.*, pp. 10-16.

[2] K. Kisskalt and M. Hartmann, "Praktikum der Bacteriologie und Protozoologie." Jena: G. Fisher, 1907.

[3] See F. Verdier, "Les Leishmanioses." Paris, 1908, p. 89, with plates.

[4] M. Miyajima, "On the Cultivation of a Bovine Piroplasma," *Philippine Journal of Science*, vol. ii, 1907, pp. 83-91.

between the tenth and fourteenth days. They are considerably more slender than the flagellate forms cultivated upon a citrate of soda medium by Kinoshita, and for this reason approximate more closely to the *Leishmania donovani* type. They differ from it, however, in the possession of an undulating membrane which completes their resemblance to the Trypanosomes. They multiply, in the same way as other Flagellates, by longitudinal fission. At room temperature they remain active for forty-five days; at a temperature of 10° to 20° C., up to three months. As with Novy's method, secondary cultures may be made by inoculating fresh blood-bouillon with material taken from the first culture. It was found possible to infect a calf with *Babesia* by inoculation with pure culture parasites.

(4) Transmission by Blood-sucking Animals.

There is no doubt that, with but few exceptions (as *Trypanosoma equiperdum*, which is transmitted by coition), blood parasites are transmitted by means of blood-sucking animals, although with many species the exact manner in which this takes place is not known. Two different types of infection are, however, distinguished.

(1) *A true change of host*, the blood-sucking animal being the host of the parasite, which can only be transmitted to a second or intermediate host after the completion of a certain stage in the development of the parasite. An instance is afforded by the conveyance of the malaria parasite from the mosquito to man.

(2) *A mechanical infection*, by the agency of a blood-sucking animal in which the parasite does not undergo further development and which is, therefore, not its true host, but which is, nevertheless, able to convey infection when its sucking or intestinal apparatus retains traces of infective material with which it has come into recent contact. Thus, *Spiroschaudinnia recurrentis*, the organism of relapsing fever, is carried by bed-bugs and rat-lice. It would appear from recent experiments that the Trypanosome of sleeping sickness is conveyed to man by *Glossina palpalis*, its true host being probably *G. fusca*.[1]

All blood-sucking invertebrates convey blood parasites, or are, at the least, seriously suspected of so doing. The following groups have come within the author's somewhat limited experience.

Mosquitoes and gnats are the true hosts of a large number of blood

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[1] See E. A. Minchin. "Investigations on the Development of Trypanosomes in the Tsetse-flies and other Diptera," *Quarterly Journal of Microscopical Science*, vol. lii, 1908, pp. 159-260.

cell parasites of mammals and birds. Of these, *Anopheles* are the most important, as they are the agents by which malarial infection is conveyed to man; while *Culex* is the host of those varieties of *Hæmoproteus*, *Proteosoma* and *Leucocytozoon*, which are parasitic in birds. *Stegomyia fasciata* carries, probably as the true host, the virus of yellow fever, and different varieties of *Culex* transmit the Filaria of man, the dog, and probably also those of birds.

Stinging-flies convey pathogenic Trypanosomes both mechanically and in the character of the true host. Such are *Glossina palpalis* and *G. fusca*, which transmit the sleeping sickness of man; and other varieties of tsetse-fly, as well as probably *Stomoxys* and the Tabanides, all of which convey the Trypanosomes of the domestic animals.

Louse-flies (Pupipara) probably carry the blood parasites of certain mammals and birds. Varieties of *Lynchia* transmit the *Hæmoproteus* of pigeons; the parasites peculiar to the bat are probably all conveyed by Nycteribia; while *Hippobosca rufipes* is believed to be the carrier of the *Trypanosoma theileri* of cattle.

Fleas, as far as our present scanty knowledge goes, can be regarded only as occasional mechanical agents of infection. As such, however, they have a considerable experimental interest, especially since the recent discovery that fleas which infest rats convey, not only the Trypanosomes of rats, but other species of Trypanosomes with which the rat has been artificially infected.

Lice may convey different serum parasites. According to Prowazek, the rat-louse, *Hæmatopinus spinulosus*, is not only the true host of the rat but, owing to its voracious habits, it may also transmit the Trypanosome mechanically. It frequently sucks more blood than it is able to digest; consequently, the next time it feeds, the slight contraction of the muscular structure of the body necessary to start the act of sucking, is sufficient to expel the undigested blood into the fresh wound. Advantage has been taken of this fact to perform experiments which have led to interesting discoveries. For instance, Manteuffel has recently proved that *Spiroschaudinnia recurrentis*, after being transmitted by inoculation to rats, may be mechanically conveyed by rat-lice, without the louse being in any sense the actual host of the parasite. It is interesting to note in this connection that the Indian Spirochæta fever of man, which resembles but is not identical with relapsing fever, is conveyed by lice.

Bugs appear to play but a small part in the transmission of blood parasites. They may, by mechanical means, convey the organism of relapsing fever, but after a prolonged sojourn in the stomach of the bug the Spirochætæ become digested (Nuttall). According to

Patton, the kala-azar parasite, *Leishmania donovani,* is conveyed by *Acanthia rotundata.*[1]

Ticks are important as being the vehicle by which *Babesia* are conveyed. In Germany, *Babesia bovis* is conveyed to cattle by *Ixodes ricinus.* Ticks are particularly interesting as being the transmitters of Hæmogregarines, experiment having proved *I. ricinus* to be the true host of *Carolysus lacertarum.* Many forms of Spirochæta infection are conveyed by ticks. Such are the African tick-fever of man, caused by *Spiroschaudinnia duttoni,* and those forms of Spirochæta infection which have been artificially induced in cocks and hens.

Leeches undoubtedly convey blood parasites to tortoises, amphibians and fishes, although definite information as to the actual species transmitted is wanting.

When experimentally inducing a parasitic infection of the blood, it is before all things necessary to ascertain by repeated blood tests that the animal to be experimented upon is free from all previous taint. Theoretically, the blood-sucking animal by means of which parasites are to be conveyed from the infected vertebrate to a fresh host, should also be examined. In practice, however, this is not possible, as in the greater number of cases the requisite proof could only be furnished by the section and microscopic examination of the body of the intermediate host. Such would be the case, for instance, with a mosquito containing malaria parasites. Attempts have been made to overcome this difficulty by using invertebrates which have been cultivated from the egg, or at least from the larval stage, in the laboratory. But the method is successful in certain cases only (as, for instance, mosquitoes which are to be infected with Proteosoma, or the parasites of human malaria), as many blood parasites are transmitted to the eggs of their host and infection is thus congenital. Such is the case with *Babesia* in ticks, *Hæmoproteus noctuæ* in *Culex,* and *Hæmogregarina stepanowi* in *Placobdella.* Speaking generally, the most that one can do is to employ as many subjects for experiment as possible; to follow the development of the parasites in the body of the blood-sucking host; and to guard against error by careful observation of control subjects. Every now and then it may be possible to procure animals for experiment from districts where the particular Protozoon under observation is either absent or very rare.

We shall now give minute instructions for the microscopic ex-

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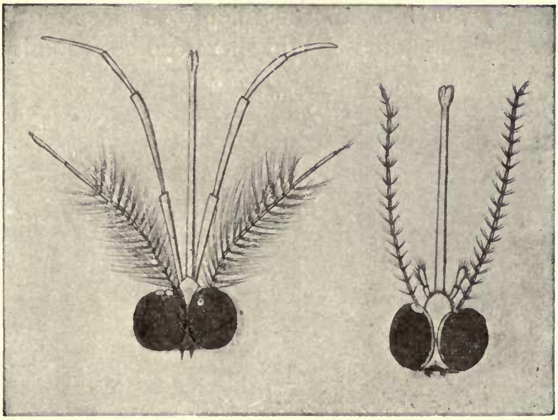
[1] W. S. Patton, "Preliminary Report on the Development of the Leishman-Donovan Body in the Bed-bug," "Scient. Mem. by Off. of the Med. and Sanit. Dep. of the Governm. of India," No. 27, Calcutta, 1907, 4to, p. 19. W. S. Patton, "The Development of the Leishman-Donovan Parasite in *Cimex rotundatus,*" Second Report, *ibid,* No. 31. Calcutta, 1907, 4to, p. 26.

amination of mosquitoes, as these, and especially the genera *Anophelina* and *Culicidæ*, have a great practical importance.[1]

In the first place, the females, which alone have an experimental interest, should be carefully distinguished from the males, and the different species should be distinguished from one another. The most minute attention must be paid to the appearance of the palpi and antennæ. In the male, the antennæ are covered with long bushy hairs, in the female with short bristles. The palpi of the female *Culex* are much shorter than the proboscis, while in the female *Anopheles* they are the same length as the proboscis, and thus a little longer than the antennæ (figs. 9 and 10). Various methods of



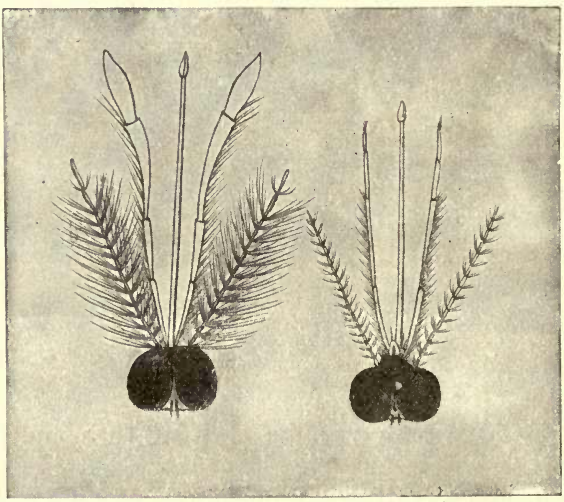
a *b*

FIG. 9.—Head of *Culex*. *a*, Male; *b*, female. (After Giles, from Braun.)

catching the flies have been recommended, but the simplest apparatus is perhaps the following: A simple glass tube, about 15 cm. in length and 2 cm. in diameter, is fitted with a cork in which a hole has been bored. Into this hole a small funnel, the external diameter of which is about 6 cm., while the lumen of the tube should measure about 5 mm., is introduced in such a way that the small end, which should be cut off diagonally, projects some 2 to 3 cm. into the lumen of the glass tube. The apparatus is inverted over the insect when at rest upon a wall (or other plane surface), and a flat disc of suitable size is inserted between the mouth of the funnel and the wall. This disc should be of metal and should be attached to the cork by means of a fine chain. On being disturbed, the insect flies up and soon passes from the funnel into the glass tube. As, like the eel in the eel-pot,

[1] Directions for the examination of other blood-sucking invertebrates (leeches, ticks, insects) are given in the final chapter of this volume.

it fails to find the opening by which it entered, the disc may be removed from the funnel and the apparatus employed to catch other specimens. Provided that they are kept sufficiently damp, mosquitoes when caught may be kept for months in glass tubes closed with gauze. V. Wasielewski describes a form of glass which appears to be very useful for this purpose. It is a small glass cylinder, closed at one end with a gauze cap, which is fixed by means of gum to a thickened rim in the glass; at the other end it is closed by a cork in which a hole has been bored. Into this hole a small glass tube is fitted which must not project inwards beyond the cork. It is furnished with a small sponge which is kept wet. The mosquitoes are fed with blood through the gauze cap. *Culex* takes three days to digest blood and then feeds again; *Anopheles*, which is more difficult to keep, is ready to feed again on the second day, and should therefore be fed daily.



a *b*

FIG. 10.—Head of *Anopheles*. *a*, Male; *b*, female. (After Giles, from Braun.)

It is sometimes expedient to cultivate mosquitoes from larvæ. They are found on damp ground near water, the *Culex* in large or small numbers, the *Anopheles* generally singly. Morphologically, they are distinguished from one another by the structure of the respiratory orifice, situated in the 8th abdominal segment. In *Culex* it is placed at the extremity of a long breathing tube or syphon, while in *Anopheles* no such breathing tube is present, the respiratory orifice being situated in a minute depression upon the exact margin of segments 8 and 9. The larvæ are best reared in small aquaria, which should be closed with gauze and protected from the sun and

dust. In their internal arrangements, these aquaria should reproduce as nearly as possible the natural conditions under which the larvæ are found.[1]

Parasites in various stages of development are found in the mid-gut and in the salivary glands of the mosquito. To examine these the insect should be prepared as follows :—

Anæsthetize with ether vapour and, after removing the wings and legs, place the insect upon a glass slide upon a dark background ; add a drop of normal saline solution. With a sharp knife separate the abdomen from the thorax in the 1st abdominal segment (fig. 11, arrow *A*). Pins should be placed at the points in the figure marked respectively *a*, *b*, *c*, and *d*, and, by careful pulling, the attachment between the 6th and 7th abdominal rings should be released. The 1st abdominal ring is held firmly by means of a pin through point *e*, and, by carefully pulling away the 7th segment by means of the pin through point *c*, the abdominal viscera (intestines and sexual apparatus) are drawn out and may be directly examined in the fresh state. The so-called "cysts" of the malaria parasite will be seen in the wall of the mid-gut (fig. 12, *m*), and the Protozoa which inhabit the lumen (the oökinets of the malaria parasite, intestinal Flagellates) may be examined through the thin intestinal wall without further preparation. Many of these, however, pass out with the bowel contents when the gut is divided.

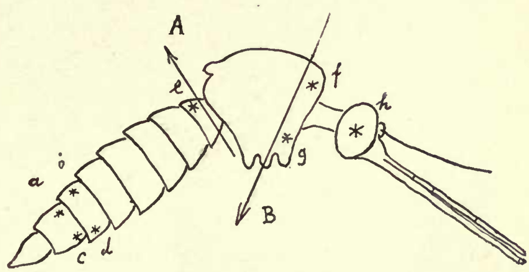


FIG. 11.—Diagram illustrating the method of preparing a mosquito for the microscope. (After Eysell, from *Mense's Handb. d. Tropenkrankh.*)

To dissect out the salivary glands a preparation microscope should be used. Press the neck forward by squeezing the thorax, and then sever the anterior portion of the thorax by a cut in the direction shown by the arrow *B*, fig. 11. With the assistance of two pins inserted at *f* and *g*, the neck is split lengthwise as far as the

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[1] Detailed instructions for rearing and keeping mosquitoes are given by Eysell, "Die Stechmücken," *Mense's Handb. d. Tropenkrankh.*, vol. ii, pp. 44-81 (Leipzig, 1905) ; by v. Wasielewski, "Studien u. Microphotogramme zur Kenntnis der pathogenen Protozoen," part 2 ; "Untersuchungen über Blutschmarotzer," 8vo, pp. 175 (Leipzig, 1908) ; by R. Blanchard, "Les Moustiques, Histoire naturelle et médicale," 8vo, pp. 673 (Paris, 1905) ; and others.

attachment of the head. If the head is now held by a pin at point *h*, the two triple-lobed salivary glands (fig. 12, *gl. sal.*) may be removed with a second pin from the loose connective tissue surrounding them and, after severing the excretory ducts, they may be examined for malaria parasites. This manipulation requires care and a little practice. The anterior stomach (fig. 12, *prov.*) is prepared in a similar fashion; its folds may harbour numerous *Hæmoproteus* in the Trypanosome stage, which will be found attached to the walls.

For permanent preparations, the mid-gut should be slightly teazed out upon a cover-glass and fixed as a cover-glass preparation in alcoholic solution of mercuric chloride. Stain with hæmatoxylin or iron-hæmatoxylin. The latter produces very pretty effects with the older cysts of the malaria parasite, if the specimens are sufficiently

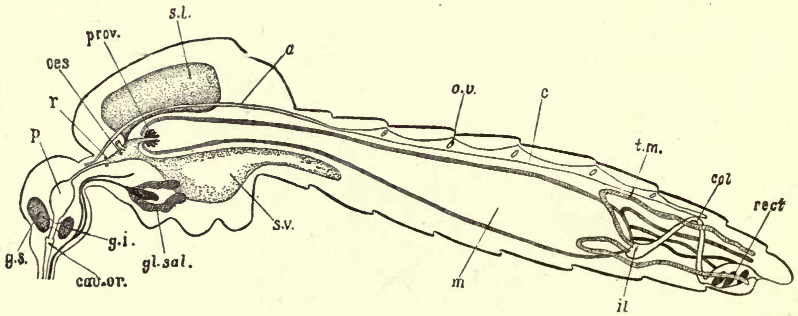

FIG. 12.—Diagrammatic illustration of the digestive and circulatory organs of a female mosquito. The mouth-parts are omitted. (After Lühe, from *Mense's Handb. d. Tropenkrankh*). *a*, Aorta. *c*, Heart. *cav. or.*, Oral cavity. *col.*, Colon. *g.i.*, Lower pharyngeal ganglion. *g.s.*, Upper pharyngeal ganglion. *gl. sal.*, Left salivary gland (the excretory duct only of the right salivary gland is shown). *il.*, Ileum (commencement of mid-gut). *m.*, Mid-gut or stomach. *œs.*, Œsophagus. *o.v.*, Venous orifices of the heart. *p.*, Pharyngeal suction apparatus. *prov.*, Anterior stomach (its folds may harbour numerous Flagellates). *r.*, Sphincter muscle (the proventriculus) at the junction of œsophagus and mid-gut. *rect.*, Rectum, with three papillæ of the right side. *s.l.*, Right dorso-lateral diverticulum of the suction stomach (the left is cut away). *s.v.*, Ventral diverticulum (invariably contains yeast-cells, which have sometimes been mistaken for developmental stages of parasitic Protozoa). *t.m.*, Malpighian tubes.

decolorized for the parasites to show up darkly against the pale grey colouring of the intestinal wall. Whole preparations of the salivary glands are made in the same way. Sections may be cut from the whole insect or from single organs fixed in alcoholic solution of mercuric chloride.

(5) Examination of Blood.

Blood for demonstration purposes should be drawn from the tail artery of rats, mice and lizards, after cutting off the tip of the tail;

it should be taken from the outer ear of dogs and the larger mammals; from the lobe of the ear of man rather than from the finger, as is usually the case; from a bare place on the breast of birds or, better still, from the inner surface of the wing; from the tail of tortoises; and from the back of fish.

A movable stage should be used, as by this means the entire specimen may be examined with the minimum of time and trouble. Otherwise, there is little to add to the directions for the examination of fresh material given in a previous chapter. These instructions should be minutely followed, however, as nowhere is a scrupulous attention to technique of greater importance than in the study of blood parasites. If the growth of the parasites is not watched in the living organism, but is merely deduced from dried and coloured specimens, there is danger of developmental stages belonging to different generations (schizonts and gametocytes of the malaria parasite) being mistaken for the developmental stages of a single generation. Or, again, a mixed infection may be overlooked and forms belonging to widely different species may be mistaken for developmental stages of a single parasitic variety. (As a matter of fact, both mistakes have occurred.)

Blood may be preserved in three ways :—

(1) *Dry Cover-glass Preparations.*—The blood parasites are the only Protozoa of which dry preparations can be made. Many forms keep better if they are fixed by exposure to osmium vapour for five to ten seconds before drying. They should be dried at room temperature and not by passing them through a flame. To keep, dry preparations should be wrapped in blotting paper and packed in tightly closed jars, with a small quantity of calcium chloride to preserve them from damp. They keep their colour somewhat longer in this way. Dry preparations which have not been exposed to the action of osmium vapour should be fixed before staining in 96 per cent. of absolute alcohol (time, ten to twenty minutes) and again dried. They are best coloured by the Giemsa modification of Romanowsky's stain. Giemsa solution (which is supplied ready for use by Grübler and Co., Leipzig) is employed in the proportion of one drop to each cubic centimetre of distilled water, and the mixture should be used immediately. The preparations must be placed material downwards in the stain, or they will be spoilt by precipitates. Two specimens may be stained at the same time, if one cover-glass rests with all four corners touching the bottom of the watch-glass while the other is made to float upon the surface of the fluid. The specimens will, as a rule, be sufficiently coloured in fifteen to twenty minutes, but the process should be watched under the microscope. When finished, the plasma should be stained blue and the nuclei red. If the parasites are very scanty, the blood corpuscles

should be watched, the process being complete when these have stained a deep violet. The preparations should be well syringed with distilled water, dried, and mounted in thickened cedar-wood oil (immersion oil). Canada balsam should not be used or the stain will not be permanent. On account of its convenience and the sharp contrasts of colour which it presents, this method is invaluable for preliminary examination and for diagnostic purposes. It is, however, useless for the study of minute details, the parasites becoming to a large extent spoilt by the process of drying. For the latter purpose:—

(2) *Damp-fixed cover-glass preparations* should be employed. The method of preparing them has already been described (p. 9). Still better is—

(3) *The preservation of blood in large quantities*, by allowing it to drip straight into a glass containing fixing mixture (alcoholic solution of mercuric chloride, Flemming's or Hermann's mixture). The contents of the glass should immediately be centrifugalized, the fixing mixture poured off, and the blood washed out. It is advisable to centrifugalize after each change of fluid. Damp-fixed blood should be coloured either with diluted hæmatoxylin, or with Salvin-Moore and Breinl's modification of the iron-hæmatoxylin method, thus: The preparations are allowed to remain for one hour in a mordant solution of 3½ per cent. iron-alum; they are then stained for half an hour in ½ per cent. solution of hæmatoxylin in distilled water, to which, after the solution of the colouring matter, a few drops of concentrated watery solution of lithium carbonate have been added. Differentiate with iron-alum in the usual manner.

(*b*) DESCRIPTION OF CERTAIN MORE IMPORTANT FORMS.

Although the blood parasites, as a class, are both numerous and interesting, it is the purpose of the present work to describe those forms only which are readily obtainable, and which are of practical interest to the student. For further information, the special textbooks of the subject should be consulted.

(*a*) *Trypanosomidæ.*

These parasites are free-living in serum. When mammalian blood is not available, fresh material may be readily obtained from certain fish. Crucians, tench and carp are the most frequent hosts. The parasites are less prevalent in winter (November to February) than in summer; and they are more frequently found in second summer carp than in first (Keysselitz).

Two varieties of Trypanosomidæ are met with in fish. Of these, one, the *Trypanosoma*, is furnished with a single flagellum attached to an undulating membrane, and closely resembles the Trypanosome of mammals (fig. 13). The second, *Trypanoplasma*, is generally the more numerous. In it the undulating membrane is prolonged into a short flagellum at the hinder end of the body, and the organism is furnished with another long and quite free flagellum (fig. 14). These parasites are easily recognized, even with a low-power lens, by their active darting movements, by which the red cells in their neighbourhood become displaced.

When examining fresh material, the student should first note the characteristic movements, which differ somewhat in the two varieties, and should then endeavour to make out something of the structural peculiarities. In the *Trypanoplasma* the flagella are placed close

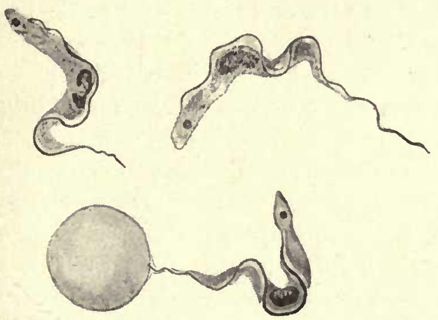

FIG. 13.—*Trypanosoma gambiense*, Dutton, from the blood of man. Magnified about 1,700 : 1. (After Dutton, from Braun.)

FIG. 14.—*Trypanosoma cyprini*. Four examples between red corpuscles from the blood of carp. (After Plehn and Hofer, from Braun.) The long free flagellum is placed at the anterior end of the parasite.

together at the anterior end of the organism; they proceed from two contiguous basal structures, behind which lies a long spindle-shaped flagellar nucleus. The round principal nucleus lies also in the front part of the body, opposite to the flagellar nucleus. In the *Trypanosoma*, however, the principal nucleus generally lies in the middle of the body, while the flagellar nucleus, which is a good deal smaller in size, is placed near the non-flagellate end. Close to the flagellar nucleus, but not always easy of detection, lies the basal structure (fig. 15) of the one flagellum, the latter forming the thickened edge of the undulating membrane. The Trypanosomidæ are characterized by the possession of eight fibrillæ or "myonemes," which run lengthwise of the flattened body, and of which four are arranged on each side. They are embedded in the thickened outer layer of plasma, and, like the basal structure, they are generally difficult of detection. This

outer plasm is called the periblast; it stains a light pink after prolonged immersion in Giemsa's stain. In cover-glass specimens, single parasites are sometimes found, which have become crushed during preparation in such a manner, that the thin inner plasma escapes through rents in the periblast. This is sometimes observed in *T. theileri* (the giant Trypanosome of cattle, which may attain a length of 0·06 to 0·07 mm.), and is also seen, though with less frequency, in smaller varieties. In these crushed examples, the myonemes are readily made out by reason of their darker colour, though their position may be very much altered by the rupture of the periblast. The term "myoneme" is misleading in this case, as the fibrillæ are not contractile, but elastic, resembling the axis-fibrillæ.

FIG. 15. — Diagram of the organization of *Trypanosoma lewisi.* (After Prowazek, from Braun.) The axis-fibrillæ are removed; the "myonemes" are dotted in.

Fission forms are not observed with any frequency among the parasites of fish, but they are frequently found in rats and mice when the infection is very severe.

Fission takes place longitudinally, the principal nucleus and the flagellar nucleus dividing independently of one another. From the flagellar nucleus of one of the daughter individuals a new flagellum is formed. This lies so closely to the original flagellum, which is retained by the second daughter individual, that its presence can be detected only after very careful examination. At a first glance it looks as if the flagellum divided from its base upwards. Colouring by Giemsa's method seems to show that the division of the principal nucleus takes place, as in the Coccidia, from within outwards by the division, in the first instance, of an inner body or caryosome (Prowazek, Lühe). But careful study of damp-fixed cover-glass specimens coloured with iron-hæmatoxylin shows that this effect is artificially produced by the Giemsa stain, and that division takes place as a result of a true mitosis (Rosenbusch [1]).

It has already been stated that Trypanosomes may be kept alive for a certain time outside the body of their host. Their examination is considerably facilitated by the fact that they may be cultivated by Novy and McNeal's method upon blood-agar.

[1] Short prefatory note in *Arch. f. Protistenkunde,* vol. xii, 1908, parts 1 and 2, pp. 169.

A small quantity of agar-agar, as used for bacteria cultures, is melted in test-tubes in a hot-water bath at a temperature of 50° C. To this is added an equal quantity of fresh defibrinated rabbit's blood. The mixture should be well shaken, and any bubbles which may have formed are punctured with a hot platinum needle, as, otherwise, they might hinder the condensation of water. The tubes are cooled in an oblique position, closed with a rubber cap, and placed for twenty-four hours in a thermostat at a temperature of 37° C. At the end of that time water will be found to have condensed freely, and tubes which have escaped complete sterilization may be recognized and removed. Blood containing Trypanosomes should be taken with a Pravaz's syringe, under antiseptic precautions, from an artery of the infected animal and mixed with a small quantity of normal saline. The skin at the site of operation should be carefully prepared. The animal may be chloroformed without endangering the success of the experiment. Three "looplets" of the infected blood should be transferred to the condensation water in one of the test-tubes, or three droplets may be conveyed by means of a sterile pipette.

These cultures are very successful in the case of the Trypanosomes of fishes and of rats, all those varieties, in fact, which do best in the refrigerator. At the end of a few days numerous Trypanosomes are seen, which agglomerate into rosettes and multiply very rapidly. Animals may be inoculated from these cultures or secondary cultures may be made. Each culture is, as a rule, infective for about one month. The culture Trypanosomes differ from those living normally in the blood-stream in the greater delicacy of certain portions of their structure (position of the flagellar nucleus, texture of the protoplasm). These modifications may be attributed, at least in part, to degenerative changes due to the abnormal medium. The tendency to agglomerate into rosettes is also a sign of degeneration.

The tsetse Trypanosome, *T. brucei*, is considerably more difficult of cultivation than the Trypanosome of the rat. Novy and McNeal recommend the following medium, though they did not find it invariably successful :—

The extract from 125 gr. beef in 1,000 c.cm. distilled water.

Agar-agar	20 gr.[1]
Peptone	20 gr.
Common salt	5 gr.
Normal (5·3 per cent.) soda solution	10 c.cm.

Of this mixture 1 part by volume is added to 2 parts defibrinated rabbit's blood at a temperature of 55° to 60° C.

[1] Nocht and Mayer found that the medium set better with 25 gr. agar-agar.

Rat-lice, the undoubted carriers of *T. lewisi,* may, on account of their flat form and their transparency, be examined for Trypanosomes under the microscope without preparation. The Trypanosomes may be exposed by isolating the intestinal canal with two fine pins, and then teasing it out in a little normal saline solution.

(*b*) *Hæmoproteus.*

A peculiar form of parasite is found in the blood of certain birds, especially singing birds, and of the carnivori. It feeds upon the red blood corpuscles, and may be either sickle-, dumb-bell-, or bean-shaped. The full-grown parasite is so placed in the long diameter of the red blood corpuscle that it encloses the nucleus with its concave side (fig. 16, *f*) without, however, as a rule, displacing it. These

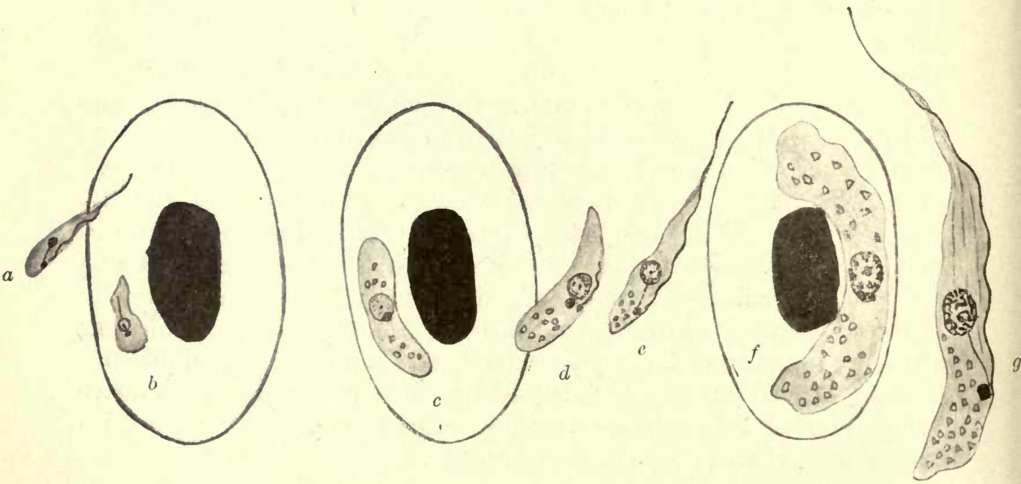

FIG. 16.—Diagrammatic representation of the changes of the asexual forms of *Hæmoproteus noctuæ* (Celli and Sanfelice) in the blood of the owl. (After Schaudinn, from Braun.) *a*, Penetration into a red blood corpuscle. *b*, Resting stage. *c*, Resting stage forty-eight hours later. *d*, Leaving the red blood cell; commencing formation of flagellum. *e*, Trypanosome form, resulting from *d*, beginning to penetrate a red blood corpuscle. *f*, Resting stage, five days after *a*. *g*, Full-grown Trypanosome.

organisms are termed *Hæmoproteus* and their significance is twofold. In the first place, it is quite certain that the process of fertilization in *Hæmoproteus* is identical with that of the other Plasmodia (malaria parasite of man and *Proteosoma*), while it may be observed under the microscope with far greater accuracy and ease. In the second place, observations of a variety of *Hæmoproteus* found in the common brown owl (*Athene noctua*), have established the fact that these non-flagellate

parasites of the red corpuscles have a developmental relationship with free-moving forms of the true Trypanosome type.

The best subject for a first examination is *Hæmoproteus noctuæ*, nearly always present in the common owl. It is found with almost equal frequency in other species of owl, as well as in the kestrel and goshawk, and is present in singing birds (finches, larks, thrushes, crows, shrikes, &c.), though infection is here less to be relied upon.

Typical free-moving Trypanosome forms are difficult to find, but the cell-parasitic, non-flagellate stages are very common. Of these, the following modifications have been described :—

(1) Asexual forms, which are capable of a certain amount of amœboid movement, and may, consequently, be more or less irregular in outline. They are sickel-, dumb-bell-, or bean-shaped, and throw out conspicuous knob-like projections at the poles. These are sometimes frayed out and the organisms may then be mistaken for fission forms. That the knob-like processes do not represent the first stage of commencing new organisms, but are merely protoplasmic processes endowed with amœboid movement, is proved by the absence of anything in the nature of nuclear division.

(2) Sex forms (gametocytes), which are incapable of amœboid movement and in which the outline is more regularly bean-shaped, with equally rounded poles. Among these, two forms are distinguished :—

(*a*) Female sex-forms (macrogametocytes), with a very much granulated protoplasm, which appears dark in colour. It takes on a comparatively darker shade when stained.

(*b*) Male sex-forms (microgametocytes), with a hyaline protoplasm, pale in appearance, which stains a light colour.

Among the structural peculiarities of *Hæmoproteus noctuæ*, the most conspicuous are the pigment granules enclosed in the plasm. These are crystalline in structure and dark brown to black in colour; their presence renders the first finding of the parasites a comparatively easy matter. They are the products of metabolism, and, for this reason, are more numerous in the slow-growing gametocytes than in the asexual forms.

The pigment of *Hæmoproteus*, like that of the Plasmodia, is doubly refractive and should be examined with the aid of a polarizing apparatus. With a crossed Nicol's prism, the pigment granules appear as bright points in the dark field. A very strong light upon the object is essential.

The nuclear structure should next be carefully studied. As in the Trypanosomes, two nuclei are present. The one a large, loosely built, principal nucleus, is situated in about the middle of the body, and stains red with Geimsa's stain. The other, a much smaller structure, staining violet with Giemsa, is placed close to the principal nucleus

and corresponds to the flagellar nucleus of Trypanosomes. Both nuclei are readily seen in the asexual forms and in the macrogametes. In the microgametes, however, the nuclear structure is more difficult of recognition, and this is especially the case with the full-grown organism. The nucleus will be found to have split up into eight double nuclei, which are crowded together in the centre of the organism, and each one of which consists of a principal and a flagellar nucleus. From these, eight daughter microgametes are formed.

Free-living forms should be sought for at night in the internal organs (liver, spleen, bone-marrow) of birds, the blood of which contains asexual forms in large numbers. According to Schaudinn, the parasite projects a flagellum and assumes a Trypanosome form under the following conditions: (1) During the six days' period of growth for the purpose of migrating from one red blood corpuscle to another (fig. 16). (2) At the end of the period of growth for the purpose of reproduction, this process consisting in longitudinal fission repeated several times in quick succession. Multiplication by rapidly repeated binary fission takes place in another species of *Hæmoproteus*, very prevalent in Brazilian doves. But in this instance it takes place, not during the free-living Trypanosome stage, but in the interior of the leucocytes, where numerous minute structures resembling *Leishmania* are formed. These are likewise observed in the internal organs only, and are most frequent in the lungs.[1]

As we have already stated, the processes of fertilization are easily followed in *Hæmoproteus*. A drop of blood containing a large number of parasites should be diluted with a little water and examined immediately, either as an ordinary cover-class preparation or in the form of a hanging drop.

The blood should not be diluted to such an extent that only single corpuscles are left in the field of vision, or the parasites will become damaged by the addition of so large a quantity of water. Manson says that it is sufficient to breathe well on the cover-glass before applying the drop of blood. The right degree of dilution is easily judged, however, after a little practice. Instead of water, Hartmann uses one part serum and nine parts 0·6 per cent. normal saline solution, one drop of the mixture being added to each droplet of strongly infected blood. The real importance of this dilution is, obviously, the change which it brings about in the osmotic conditions. If undiluted blood is examined under conditions which permit of the access of air, the phenomena of fertilization will take place as the result, apparently, of the slow evaporation of water. But if undiluted

[1] H. de B. Arago, "Ueber den Entwickelungsgang und die Uebertragung von *Hæmoproteus columbæ*," *Arch. f. Protistenkunde*, vol. xii, parts 1 and 2, 1908, pp. 154-167.

blood is examined under conditions which do not permit of the access of air, these phenomena will not be observed.

Temperature conditions are also a factor of importance. A temperature of about 20° to 25° C. is the most favourable; temperatures of about 18° C. or under, and 30° C. or over, are unfavourable.

The first point to observe in the finished specimen, is the way in which the gametocytes become rounded and escape from the red corpuscles by bursting them open. In the microgametocytes, active streamings of the protoplasm commence, which cause the knoblike plasmic process to appear and disappear, and which lead to the rapid formation of microgametes. These spring suddenly as long hyaline threads, four to eight in number, from the body of the parasite. They immediately commence violent thrashing movements, by which they finally succeed in freeing themselves from the parent organism, when they writhe away. In the meantime, though this is not so easily followed, a change has taken place in the macrogametocytes. This

a *b* *c* *d* *e*

FIG. 17.—Final stages of development of the sex-forms of the parasite of human malignant malaria (*Laverania malariæ* or *Plasmodium immaculatum*). *a*, *b*, Female; *c*—*e*, male.

consists in the reduction of the nucleus, by which the macrogametocyte becomes a sexually mature macrogamete. The macrogamete is now fertilized by the agency of a microgamete. The copulation of the two sexual forms is followed by a period of rest. Then, at a spot upon the surface of the new individual, a conical protruberance begins to appear, which gradually increases in size and from which, after a quarter to half an hour, the long oökinet proceeds, which moves by means of writhing movements forwards. The entire process may be observed inside three-quarters to one hour (figs. 17 and 18, *13* to *17*).

The reproductive process can also be followed in fixed and coloured specimens. A number of glass slides are prepared with diluted blood, as described above, and placed in a damp chamber. They should be fixed singly and at suitable intervals.

Under normal conditions the fertilization of *Hæmoproteus noctuæ* takes place in the stomach of *Culex pipiens*. The artificial infection of the mosquito is successful, however, in only a small proportion of cases, and the operation requires so much time and patience that the method is of little use, either to the student or for purposes of

demonstration. We shall, for this reason, omit any description of the complicated developmental stages which take place within the stomach of the mosquito. It is sufficient for the present purpose to state that the oökinets speedily develop flagella and assume the typical Trypanosome form.

According to Hartmann, *H. noctuæ* may be cultivated similarly to *T. lewisi* upon blood-agar (p. 59). In the first twenty-four hours, single oökinets appear in the cultures. In the next few days, flagellate forms appear, similar to those in the gut of the *Culex*.

Note : *Leucocytozoon ziemanni.*

When examining the blood of owls or other birds of prey for *Hæmoproteus*, it is by no means unusual to come upon the sex-forms of *L. ziemanni.* In a pale spindle-shaped envelope, which corresponds to the modified periblast, and which is of about the same length as a red blood corpuscle, lies an oval plasmic body, which exhibits the same dimorphism in regard to structure of the plasm and nuclear conditions as the sex-forms of *Hæmoproteus.* In its immediate vicinity is seen the nucleus of a red corpuscle, deformed to a dumb-bell shape by the depredations of the parasite. The parasite is of comparatively large size, and for this reason the eight double nuclei in the microgamete stage are more easily recognized than in *Hæmoproteus.*

(*c*) *Babesia.*

The theoretic value of *Babesia* approximates to that of *Hæmoproteus*, while its practical importance is considerable. Unfortunately, fresh material is difficult to obtain, but blood smears containing *Babesia canis* should be procured if in any way possible, as their examination is very instructive. The parasites will be found occupying the red corpuscles, and their nuclear structure should be carefully studied. Although the flagella are absent, two nuclei are present, as in the Trypanosomes. The larger or principal nucleus stains pink with Romanowsky, while the smaller or flagellar nucleus takes on a more violent tone. The latter is readily discernible, as it is placed close to the pointed end of the more or less pear-shaped parasite. The formation of a flagellum from this nucleus has been observed only in cultures upon artificial media.

(*d*) *Plasmodia.*

Opportunities of examining the malaria parasite in the living state are rare in this country, but cover-glass specimens may sometimes be obtained. Multiplication by schizogony is usually synchronous in

all the parasites present in the blood, and takes place shortly before the commencement of a feverish attack. For this reason, the life-history of the malaria parasite is to be followed only by examining specimens of blood taken at different stages of the disease.

A species of Plasmodium which is valuable for purposes of comparison, or it may even be used for demonstration in place of the malaria parasite, is *Proteosoma præcox*, found in the blood of indigenous singing birds, though it is less frequent than *Hæmoproteus noctuæ.* It should be sought in sparrows, these birds being easily procurable in large numbers. Frequency of infection varies with the season and with the locality. Ruge found that from April to September was the most favourable period in Berlin, nearly 30 per cent. of sparrows being infected. Demonstration is rendered difficult by the fact that, after a short acute stage of infection, the parasites become so few in number that their presence cannot be proved by direct examination of the blood, but only by inoculation of control birds. *Proteosoma* may, however, be transmitted to canaries, and this factor is of considerable assistance in maintaining a stock of parasites once they have been found.

The developmental stages are shown in fig. 18. The differentiation of the two sex-forms, both from one another and from the asexual form, is similar to that observed in *Hæmoproteus.* The pigment granules in the plasma also resemble those of *Hæmoproteus* and should be examined in the same way. The maturing of the sex-form may also in this case be watched under the microscope, but the Plasmodida do not attain to the oökinet stage under artificial conditions.

It will be sufficient for the present purpose to describe those features in which the species vary and to which special attention should be paid by the student.

The *Proteosoma* of birds differs from the human malaria parasite in that, shizogony not being synchronous, different developmental stages may be observed in a single blood preparation. It manifests, moreover, a double nucleation similar to that of *Hæmoproteus*, having a large principal nucleus which colours pale pink with Giemsa, and close to it a smaller nucleus which stains a dark violet and which corresponds to the flagellar nucleus of the Trypanosomes (fig. 19, *b*). At certain developmental stages, *Proteosoma* are furnished with a flagellum similar to that of the Trypanosomes (fig. 19, *a* and *c*).

Of the human malaria parasites, the germ of malignant fever, *Laverania malariæ,* is distinguished from the others by its immature sex-forms, which have a characteristic crescent shape. (fig. 17). The benign tertian parasite (*Plasmodium vivax,* fig. 18) causes the affected erythrocyte to swell until it becomes larger than the normal. It forms 14 to 24 (generally 16) merozoites, and the entire developmental

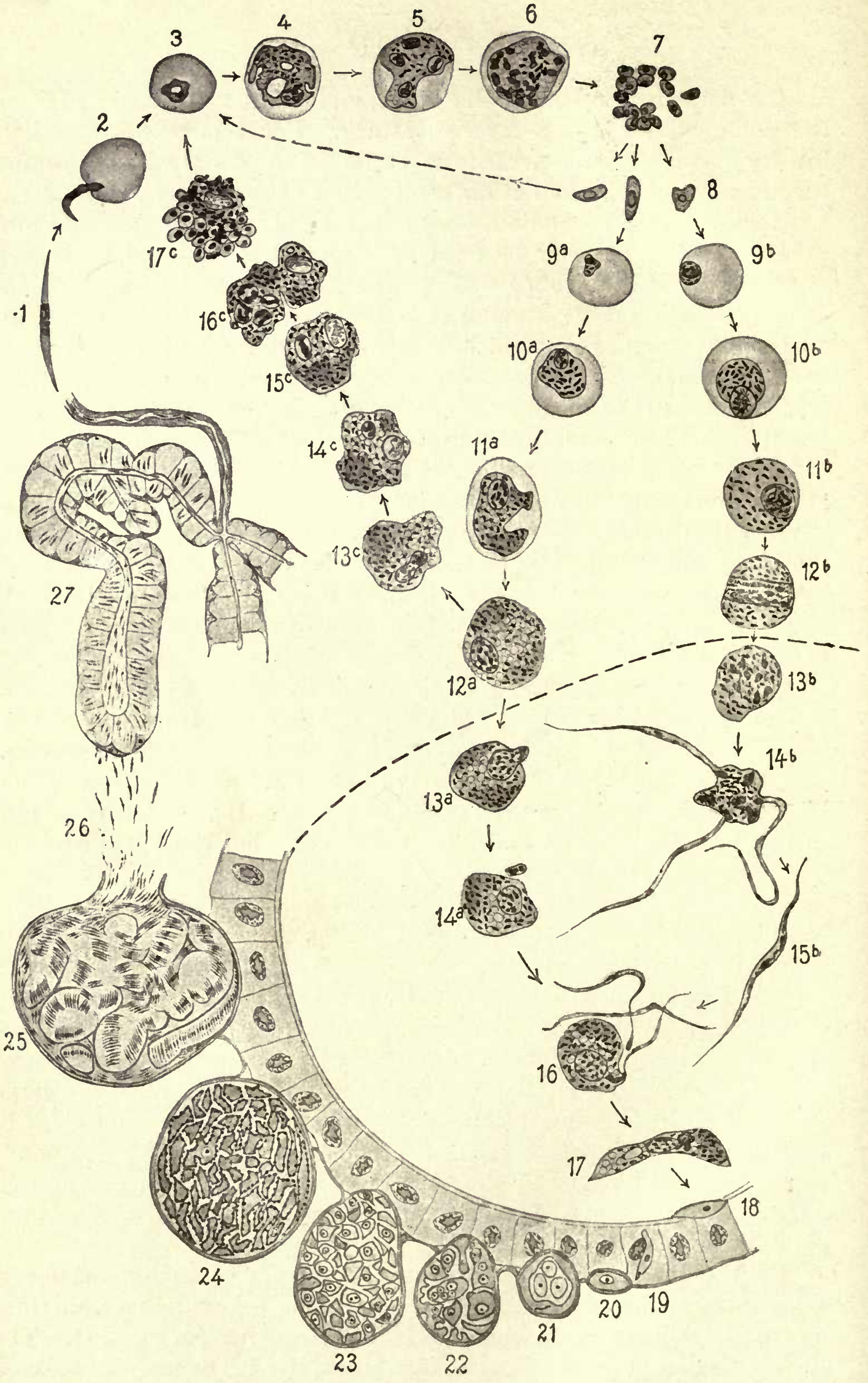

FIG. 18.—Diagram to illustrate the life-history of the human tertian parasite, *Plasmodium vivax.* (After Lühe, from Braun.) Magnification: *1—17*, 1,200 : 1. *18—27*, 600 : 1. *1*, Sporozoite. *2*, Penetration of the sporozoite into a red blood cell. *3—4*, Growth of the

process of each asexual generation is completed in forty-eight hours. The parasite of quartan fever (*Plasmodium malariæ*) is smaller in size; it does not cause the red blood corpuscles to swell; it forms only 6 to 12, and generally 8, merozoites; and the asexual form takes seventy-two hours to complete its cycle.

To cultivate the stages which develop in the mosquito, a temperature of 25° to 30° C. must be maintained. Human malaria germs develop in *Anopheles*, and *Proteosoma* in *Culex pipiens*. A period of nine days is required for the completion of schizogony. Mosquitoes should be examined by the method already described.

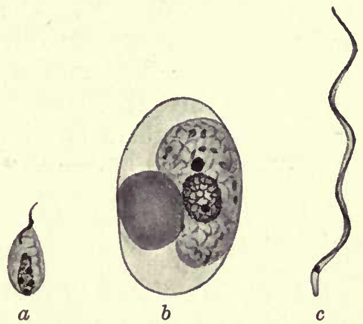

FIG. 19.—*Proteosoma præcox*. (After Hartmann.) *a*, Merozoite. *b*, Macrogametocyte. *c*, Microgamete. Magnified about 2,250 : 1.

Class IV. **Telosporidia.**

The class Telosporidia is composed of two orders, the Coccides and the Gregarines. They are closely related and are purely parasitic in their habits. The alternation of generation in the malaria parasite is so strikingly analogous to that which occurs in the Coccides, that the Hæmosporides have always, up to the present, been included as a third order of the Telosporidia. They differ, however, in several essential particulars from these, more especially in the absence of enclosed sex-forms and in the motile character of the copula. Enclosed reproductive bodies (sporocysts or pseudo-navicellæ), containing, rarely one and generally several, long, thin germs (sporozoites), are peculiarly characteristic of both the Coccides and the Gregarines, although the method of their development is not the same in the two orders. The *Aggregata* are usually classed as Gregarines, but it is highly probable that they should form a third order of Telosporidia, quite distinct from the other two. For this reason the description of them is appended as a note to the Gregarines.

schizont. *5—6*, Nuclear division within the schizont. *7*, Breaking up of the schizont into merozoites. *8*, Single merozoites, one of which (left arrow) enters a blood corpuscle and develops into a schizont (*3—7*). After a certain period of infection the sex-forms develop: *9a—12a*, Macrogametocytes. *9b—12b*, Microgametocytes. If the macrogametocytes remain in the blood-stream of man, they multiply by parthenogenesis and form schizonts (*13c—17c*). Below the dotted line the stages which take place in the gut of *Anopheles* are shown: *13a—14a*, Maturation of the macrogametes. *13b—14b*, Formation of the microgametes. *15b*, A microgamete. *16*, Copulation. *17*, Motile copula or "oökinet." *18*, Oökinet penetrating the intestinal wall of the mosquito. *19*, Oökinet passing through the epithelium. *20—25*, Sporogony upon the outer surface of the intestinal wall. *26*, Passage of the sporozoites to the salivary gland. *27*, Salivary gland of mosquito containing sporozoites.

Order 1. *Coccidia.*

The Coccides are typical cell parasites and, in particular, parasites of the epithelial cells. The young free-living forms and the mature male sex-forms are actively motile, the latter being furnished with special organelles of movement in the shape of two flagella. In the full-grown stage the parasites are either oval or round. The cuticle is absent and there is no definite separation of the ecto- from the endoplasm. The developmental cycle always includes an alternation of generation, but it is never associated with a change of host. Transmission to a fresh host is brought about by the agency of the "oöcysts," which are protected from external influences by a resistant shell (fig. 20, XV). Within the oöcyst, by repeated division of the soft body, termed "sporogony," a number of "sporoblasts" are formed (fig. 20, XVIII), which, in their turn, surround themselves with a secondary envelope or "sporocyst," and from which the "sporozoites" finally proceed (fig. 20, XIX). After transmission to the intestine of a suitable host, the sporozoites emerge from their covering (fig. 20, XX) and penetrate the epithelial cells (fig. 20, II), where they develop into "schizonts" (fig. 20, III-IV). The schizonts reproduce themselves by multiple division (fig. 20, III-IV), without previously encysting (fig. 20, V-VII). The products of this division are termed "merozoites" (fig. 20, VIII-X); they either develop into schizonts and so increase the virulence of infection, or, if several asexual cycles have followed one another, they become immature sex individuals or "gametocytes" (fig. 20, XI^a-XI^b, XII -XII^b). The female germ, or "macrogametocyte," develops into the mature "macrogamete" by reduction of the nucleus without cell-division. The male germ, or "microgametocyte," by a process of multiple cell-division called "gamogony," forms numerous bi-flagellate "microgametes," which emerge, leaving behind a large residual body (fig. 20, XII^c-XII^e). The fertilization of a macrogamete by a microgamete (fig. 20, XIII-XIV) is followed by the formation of the "oöcyst," which we took as the first stage in the developmental cycle. The oöcyst is formed by the secretion on the part of the copula of a protective membrane, which hardens to form the oöcystic shell; or, where the macrogamete is already furnished with such a membrane, the hole or "micropyle," through the microgamete made its entrance, merely closes.

The Coccides are parasitic in a large number of vertebrates, molluscs, and articulates. They are found in the intestine and its appendages as well as in the other excretory organs. The varieties most suitable for demonstration purposes are those found in the gut of *Lithobius forficatus*, in the kidney of *Helix nemoralis*, and in the liver of rabbits.

(*a*) *Coccidia found in the Intestine of* Lithobius.

L. forficatus, L., is a chestnut-brown centipede, about 2 cm. in length, composed of fifteen segments, each of which is furnished with a pair of long and powerful legs. It is found under the bark of old tree-trunks; it also occurs in the rotting wood of old stumps, in old

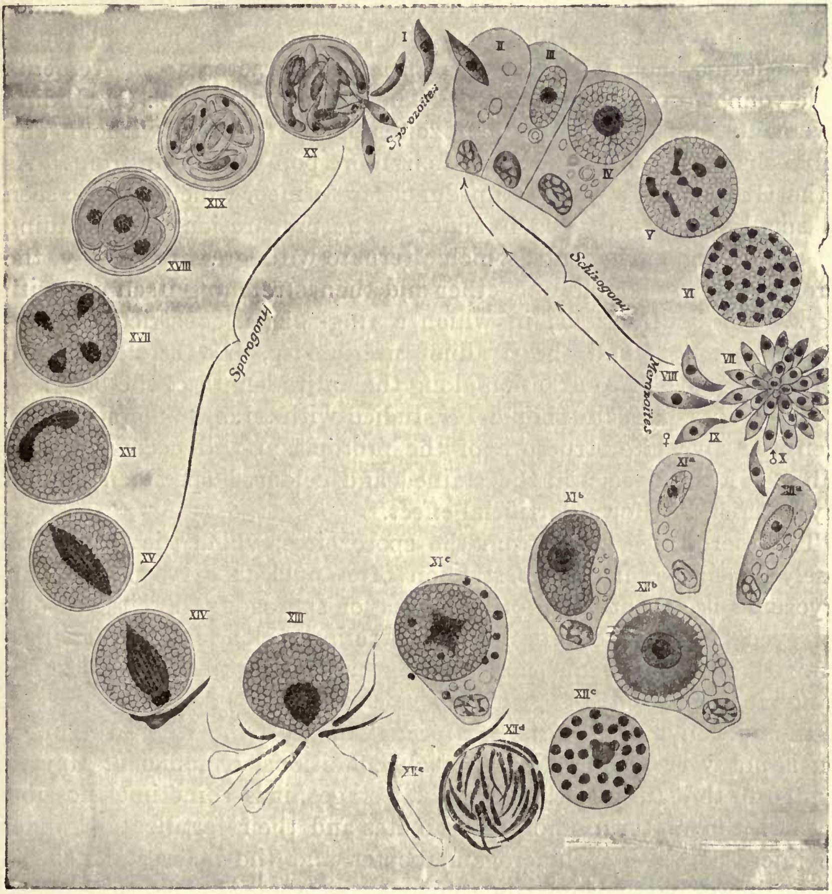

FIG. 20.—Diagram to illustrate the developmental cycle of *Eimeria schubergi* (Schaud.), from the intestine of *Lithobius*. Description in the text. (After Schaudinn, from Braun.)

compost heaps, &c.; and it is sometimes found underneath stones. Infection is most likely where centipedes are present in large numbers.

Examine for Coccides as follows: Cut off the head and tail segments, when, by the strong contraction of the body muscles, the brownish gut will be protruded from the neck orifice and may be drawn out with fine tweezers. Any portions of the fat-body attaching

to it should be removed, and the intestine (which is so long that a fourth part will be sufficient for demonstration purposes) teazed out in normal saline solution. The parasites will remain alive for a slightly longer time (two to three hours) if, instead of normal saline, the contents of the gut are diluted with the body secretion of the dead Lithobius, this generally sufficing for one preparation.

If the centipede is infected, all the developmental stages characteristic of the Coccidia will be found in the gut. The asexual reproductive forms should be noted, as also the characteristic movements of the young forms or merozoites. These movements are: (1) Gliding movements, by which the merozoites move forward without any visible change of form and with their more pointed end in front. As in the Gregarines, a jelly-like peduncle is formed, which may be seen if a small quantity of sepia is added to the preparation. (2) Bending and stretching movements, which alternate with great regularity, the merozoite slowly doubling together and then stretching itself out with a jerk. (3) Contractions, in which a ring-shaped constriction, commencing close behind the hyaline anterior tip, travels slowly as a continuous wave of contraction along the entire length of the body.

The living parasite should be studied with an oil-immersion lens, when the alveolate structure of the protoplasm will become clearly visible. If this is seen in the stained and coloured specimen only, it may be mistaken for an artificial effect.

The merozoites differ from the sporozoites, which they otherwise closely resemble, in the possession of a nuclear caryosome. This caryosome is absent in all the stages of sporogony, as it becomes destroyed in the process of nuclear reduction which precedes fertilization. It does not again make its appearance until the sporozoites change into schizonts.

In addition to schizonts, sex-forms will be found where the infection is not very recent. Under such conditions, if sufficient time is devoted to the study of the individual stages, it is sometimes possible to watch the maturation of the gametes and their fertilization under the microscope. The macrogametocytes and the macrogametes become differentiated from the schizonts by the accumulation in the plasma of granular reserve material of a high refractivity. The microgametes, on the other hand, are easily recognized by their clear, close, small-meshed protoplasm.

The identification of the different developmental stages is rendered difficult by the fact that three varieties of Coccides are found in the centipede, and they may all be present in a single host at one and the same time. Of these three varieties, one only, *Adelea ovata*, undergoes sporogony in the intestine of its host, where oöcysts in all stages of development may be found. They are oval in shape and develop

numerous "sporocysts," which have a disclike appearance. Each contains two sporozoites and a comparatively large residual body. *Eimeria lacazei* is, like *A. ovata*, of comparatively common occurrence; it is distinguished by the oval form of its schizonts. *E. schubergi*, the schizonts of which are ball-shaped, is of rarer occurrence. Both the *Eimeria*, however, vacate their host soon after fertilization; the oöcysts when formed pass out in the fæces and sporogony takes place in the open. This process may be followed by keeping the live centipedes in flat glass dishes, the bottoms of which are covered with blotting paper over which cover-glasses are laid. To keep the cover-glasses in place, well damp the blotting paper, otherwise they may be deranged by the movements of the centipedes. The droppings of infected animals are generally somewhat soft and may be readily spread out on the cover-glasses and examined.

Permanent coloured specimens are made by fixing cover-glass preparations of the intestinal contents in alcoholic solution of mercuric chloride, staining with diluted hæmatoxylin, and differentiating with eosin. The most important point for observation is the structure of the nucleus. To study the effects of the parasites upon the intestinal epithelium, fix the entire gut unopened in alcoholic solution of mercuric chloride and cut in series.

It is important to remember that, in addition to the Coccides, two large polycystic species of Gregarine are found, though not very frequently, in the gut of the centipede.

(*b*) *Coccidia found in the Kidney of* Helix.

Another variety of Coccides which is very readily obtainable is parasitic in the kidney of certain land snails. It is found in the common garden snails, *Helix nemoralis*, L., and *H. hortensis*, Muell. Its frequency varies with locality, but it is generally present in one out of several examples.

The shell should be removed from the snail in small pieces with a strong pair of tweezers, one point of which is inserted between the mantle and the shell. The kidney lies lengthwise in the largest and lowest spiral of the visceral sac, and is easily seen on account of its superficial position and yellow colour. A piece should be cut off with the scissors and teazed out in normal saline or, better still, in a drop of blood from the snail. The resulting liquid will be found to have a milky cloudiness, due to the large quantity of opaque urinary concretions. These concretions are knobbly and irregular in shape, and are frequently enclosed in isolated, but otherwise undamaged, kidney cells. The parasites, which belong to the species *Klossia helicina*, are also frequently found in the host-cells, which become much enlarged.

Both the mature forms and the motile young forms should be studied, and the oöcysts should be sought for. These, as in *Adelea ovata*, undergo the entire process of sporogony within the body of their host. They are round in shape, and the soft body, which in the unicellular stage is known as a "sporont," divides into numerous sporoblasts which, by the secretion of an envelope, become changed into "sporocysts." These are likewise round, and within them five to six sporozoites are formed, leaving a small residual body.

The early stages of the process of fertilization are more easily seen in *Klossia* than in the *Eimeria*, the copulation or merging of the gametes being preceded by a conjugation or transitory attachment of the immature gametocytes. It is only after conjugation has taken place that the microgametocyte, which is smaller than the macrogametocyte, divides into four microgametes, one of which completes the fertilization of the macrogamete, which has, in the meantime, become mature by reduction of the nucleus. A similar process is performed by *Adelea ovata* in the intestine of *Lithobius*.

Cover-glass preparations are made by passing a cut surface of the infected kidney under gentle pressure over a cover-glass. They should be fixed and stained in the same way as preparations of the Coccides of the centipede. Or thionin may be used for colouring, as it is particularly successful in showing the granulations of the protoplasm.

The examination of sections is very instructive. For these, the kidney should be fixed whole in alcoholic solution of mercuric chloride, or in Flemming's or Hermann's mixture. If the sublimate solution is used they should be coloured with hæmatoxylin, but with safranin after fixing with mixtures containing osmium. The epithelium of the kidney consists of a single layer of cells; it projects from the kidney wall into the interior of the organ in numerous folds which are supported by lamellæ of connective tissue. These folds are arranged in such a way as to occupy the hollow space in the interior of the organ. The parasites are found in the enlarged cells of the glands, and they are generally united in groups.

(c) *Coccidia found in the Liver of the Rabbit.*

The coccidiosis of rabbits is epizootic in its occurrence, and usually affects the young rabbits of a stock. As a general rule, only old healed-up knots are found in the livers of old animals. Infection is established in the case of the living animal by the presence of oöcysts in the dung. Fresh material being somewhat difficult to obtain, it is generally necessary to rely on fixed and coloured specimens.

The parasites invariably belong to one species, namely, *Eimeria stiedæ* (Lindem.), and are found in both the liver and the intestines. The changes which take place in the liver are particularly interesting.

In the liver of the rabbit, the Coccides inhabit the gall-ducts, where they cause cystic enlargement, the swellings being visible to the naked eye as largish yellow knots. In the acute condition which, when infection is severe, may end fatally, these knots are taut and elastic to the touch. They are filled with a thin pus-like liquid which should be examined under the microscope, either fresh or as a smear preparation by the method given for the Coccidia of *Lithobius*. This liquid will be found to contain numerous parasites in various stages of schizogony. When the disease has passed its height, the cyst-contents become drier and more like crumbling cheese.

The asexual forms are very numerous in the knots with liquid contents; in those with drier contents only encysted parasites are, as a rule, found.

In the living state, the developing schizonts are easily distinguished from the epithelial cells which harbour them by their greater refractivity. The multiplication of the nucleus, which precedes the multiplication of the cell, frequently commences before the end of the period of growth. The resulting merozoites are more numerous than in the Coccidia of *Lithobius* and the details of schizogony are consequently more difficult of observation. The number of microgametes formed from each microgametocyte is also greater and the size of the individuals (which are actively motile) is consequently less.

The macrogametocytes are easily recognized by the numerous granulations in the superficial layer of plasma, which, under certain conditions, may resemble small nuclei, similar to those which appear in the microgametocyte during the formation of the microgametes. The presence, however, of a large undivided nucleus in the interior of the macrogametocyte will prevent any confusion of the forms. In older growths, next to the encysted parasites, the macrogametocytes are the forms most frequently encountered.

The immature macrogametocytes are ball-shaped, while the fertilized macrogametes assume an oval form. The oöcysts, which are formed by the secretion on the part of the macrogamete of a doubly contoured envelope, are also oval in form. The next stages take place, not within the liver or intestine of the rabbit, but in the open air, oxygen being necessary to their further development. To follow these stages under the microscope, fæces or gall containing oöcysts should be spread out in the air. Material taken from growths in the liver does not yield reliable results, as oöcysts which are retained for any length of time in the liver, become injured to such an extent by the large amount of carbonic acid which it contains, that they lose their power of development and become degenerated. In the gall-bladder of rabbits suffering from coccidiosis of the liver, a fine

sandy deposit is found, which is composed entirely of numerous oöcysts. If normal oöcysts are spread out in shallow dishes with a small quantity of gall, the developmental process will be continued. The oöcysts will divide into four sporoblasts which, by the secretion of an envelope, become changed into "sporocysts," from the interior of each of which proceed two sporozoites. This developmental process, which is complete in about seventy hours, may be followed under the microscope by isolating single parasites and keeping them in a damp chamber. To prevent decomposition a small quantity of thymol may be added to the material, or it may be treated in the first instance with 4 per cent. solution of potassium permanganate; owing to the extreme impermeability of its shell, the developmental capacity of the oöcyst will remain unimpaired. The final emergence of the sporozoite from the shell is due to the influence of the pancreatic secretion. This may be proved by treating oöcysts containing perfect sporozoites with a preparation of pancreas. The best extract for this purpose is pancreatin (Dr. G. Gruebler and Co., Leipzig), a small quantity of which should be dissolved in a few cubic centimetres of water, containing just a trace of soda. Both oöcyst and sporocyst are furnished with a micropyle, which opens under the influence of the pancreatic secretion and out of which the sporozoite glides.[1] In the *Eimeria* of the centipede, the oöcyst alone possesses a micropyle through which the sporozoite escapes, while the envelope of the sporocysts is bivalved and opens under the influence of the gastric secretion.

Cover-glass preparations of the Coccidia of rabbits are made in the same way as those of the other Coccidia. It is not possible, however, to get satisfactory results with the staining of encysted parasites, owing to the extreme impermeability of the oöcystic envelope. When staining with iron-hæmatoxylin, counter-stain with Bordeaux red; by this means the nuclei will become black, while the granulations of the macrogametes, which otherwise are difficult to distinguish from the chromatic elements, are coloured red.

Sections of coccidial lesions from the liver of rabbits have a special interest, because they show the manner in which the organ becomes pathologically changed. The growths should be cut out and fixed whole in alcoholic solution of mercuric chloride, and sections should be stained singly with hæmatoxylin and eosin. It will be seen from these sections that the growths are due to pronounced local proliferation of the infected gall-ducts, which become cystically enlarged, and into the lumen of which numerous folds of proliferated wall-tissue project. The oöcysts of Coccides are found free in the interior of the

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[1] For further details, see R. Metzner, "Untersuchungen an Coccidium cuniculi," *Arch. f. Protistenkunde*, vol. ii, 1903, pp. 13-72.

growth, while the other developmental stages occur in the epithelial cells.

Old healed-up scars are also very instructive. The most striking appearance is the extreme proliferation of the connective tissue, by which the tumour contents have become destroyed. As in the case of other old parasitic foci which have become destroyed by encysting with connective tissue, calcification ensues. The only remaining traces of the parasitic invasion are the shells of dead oöcysts.

The same species of Coccidium is met with in the small intestine of the rabbit, where it is likewise parasitic in the epithelial cells, and gives rise to violent diarrhœa. Diarrhœa, in combination with emaciation and loss of appetite, is very suggestive of coccidial infection, the presence of oöcysts in the fæces establishing the diagnosis. *Eimeria stiedæ* is found not only in the rabbit, but also occasionally in man, as well as in different domestic animals (cattle, horses, goats, swine). In cattle, it usually inhabits the large intestine and the rectum, and gives rise to a disease known as red flux. The condition is accompanied by blood in the fæces, and it appears enzootically in the summer and autumn among cattle on high-lying pastures in Switzerland.

A similar parasite, *E. falciformis* (Eim.) which, like *E. stiedæ*, forms only a small number of merozoites, is parasitic in the house-mouse. It is generally found in the intestine, more rarely in the liver.

Another Coccidium, *Isospora bigemina* (Stiles) is found in the intestine of cats and dogs. The oöcyst of this parasite contain, not, as in *Eimeria*, four sporocysts with two sporozoites each, but two sporocysts with four sporozoites each.

(*d*) *Pseudo-coccidiida.*

Before leaving the subject of the Coccidia, it is necessary to issue a note of warning to the student as to the caution with which his investigations must be pursued, for there is no class of the Protozoa in connection with which mistakes are so likely to arise. It frequently happened, formerly, that stages of Coccides were mistaken for the eggs of Helminthes. Now, however, that the parasitic Protozoa are receiving so large a measure of attention, the tendency is to discover Coccides where none are present. Eggs of Nematodes and of Distoma have frequently been mistaken for Coccidia, the commonest errors being in the case of *Distoma turgidum*, which forms tumour-like swellings at the pylorus of the frog, and *D. pellucidum*, which is occasionally found in the albumen of hens' eggs. Even the calcified body of a *Cysticercus* has recently been mistaken for a

Coccidium, and this in spite of its concentric stratification and compact non-cellular structure. Similar errors have been made by reporting the presence of Coccidia in the sweat-glands of swine suffering from the so-called Schrotausschlag.[1] Errors such as these show the necessity for caution, though where the details of technique are faithfully carried out they are unlikely to arise. Schaudinn's work on the Coccidia of *Lithobius* will be found a valuable aid to the study of Coccidia in general.[2]

Order 2. *Gregarinidæ.*

Gregarines occur exclusively in the invertebrates. They are generally long, but may be oval, in shape. In their young stages they are wholly or in part cell parasites, but the older forms live free in the hollow organs (gut, body cavity). They are motile in both the young and the adult stages. Unlike the Coccides, they possess a cuticle, and there is a marked differentiation between the ecto- and the endoplasm. Their life-history rarely (Schizogregarines) includes an alternation of generation and never a change of host. The body may be homogeneous (monocysts), or it may be separated, by means of a membrane thrown out from the ectoplasm, into two divisions, which are placed one behind the other (polycysts). The best subjects for preliminary study are the monocysts of the seminal vesicles of the earthworm, and the polycysts of the intestine of the mealworm.

(*a*) *Gregarines of the Earthworm.*

The mature cysts of Gregarines are nearly always present in the seminal vesicles of large earthworms. Young cysts and the free Gregarine forms occur with less regularity, though they generally make their appearance in the spring and are rarely absent in about the month of May.

Earthworms are prepared as follows: The worm is stretched out in a shallow dish with its darker dorsal aspect uppermost. The body-tube is split at the anterior end with a sharp scalpel along the middle line, and, after separating it from the intestinal canal by severing the septa, it is turned back and pinned down. Three pairs of large whitish-yellow bladders lying between the 11th and 13th segments will then become visible. These are the seminal vesicles. A small portion should be cut off with the scissors and its contents diluted with a normal saline previous to examination.

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[1] M. Lühe, "Ueber den Schrotausschlag der Schweine und das sogenannte 'Coccidium fuscum,'" *Centralbl. f. Bakt. u. Paraskde.*, part i, vol. xxix, 1901, pp. 693-698.

[2] F. Schaudinn, "Zoolog. Jahrb.," Abt. f. Anat., vol. xiii, 1900, pp. 197.

The contents of the seminal vesicle will be found to consist almost entirely of seminal cells and mother-cells in different stages of development. Among them, Gregarine forms, both encysted and free, will be seen in large numbers, though the free forms may be absent altogether.

Several different species of Gregarine are parasitic in the seminal vesicles of *Lumbricus terrestris, L.* (also known as *L. herculeus*, Sav., and *L. agricola*, Hoffm.), the largest of our native earthworms. Of these very little is known, but it is certain that they all come under the heading of monocysts. The species most frequently encountered is *Monocystis lumbrici* (Henle), better known as *M. agilis*, Stein. On account of its extreme motility it makes a very good subject for examination. Although very small in size, measuring only about 0·2 to 0·3 mm. in length, its comparative opacity renders it easily discernible with a low power lens.

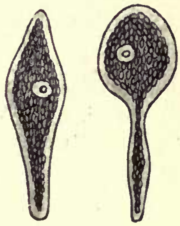

Fig. 21.—*Monocystis lumbricis* (Henle) in two different stages of movement. Magnified 250:1. (After Stein, from Braun.)

The living Gregarine shows a marked differentiation of the granular endoplasm from the hyaline ectoplasm. At the margin of contact there is a single layer of very fine, closely packed, spincter-muscle fibrillæ, while the body surface is covered with a thin cuticle in which a fine longitudinal striation is discernible.

Bending and gliding movements are rarely performed by the free-living forms. The most conspicuous motile phenomena are the contractions, which give rise to lively streamings of the endoplasm, the granulations streaming away from one end of the body, which swells out and becomes club-shaped, while the opposite end becomes proportionally thinned. Then, almost without perceptible pause, the process is reversed and the streamings run in the opposite direction, accompanied by a corresponding change of body shape. By this process, the bladder-shaped inner body which contains the nucleus is tossed from end to end, scarcely remaining stationary at all.

The first stage of reproduction consists in the association at their anterior ends of two Gregarines, which then proceed to surround themselves with a common cystic membrane. This process is rarely observed directly, but the young cysts, which are round in shape, are always to be found in the month of May. In them the two Gregarines are seen to lie side by side but quite distinct from one another; they are contracted into balls and do not touch the cystic envelope. In a later stage, each of the two Gregarines undergoes reproduction independently of the other, this process resulting in the formation from the peripheral layer of a number of little daughter-cells. In the mature cysts numerous spindle-shaped "pseudo-navicellæ" are

formed and these contain sporozoites within a somewhat resistant envelope.

The finer structural details of the plasm and nucleus are best observed in cover-glass preparations, which should be fixed in alcoholic solution of mercuric chloride and stained with hæmatoxylin. The manner in which the bladder-shaped nucleus becomes dissolved after encystment is seen in quite young cysts. The greater part of the nucleus, with the exception of the caryosome, perishes. It becomes broken up and absorbed by the plasma, a small portion of the vegetative nucleus being used to form a sex-nucleus, which divides repeatedly by mitosis. The more minute details of these nuclear changes are, however, only to be seen in sections. The pseudo-navicellæ, moreover, do not stain well in cover-glass specimens, even though the utmost care be taken to spread the cyst contents in the thinnest possible smear upon the glass.

Sections are cut from the entire seminal vesicle, which should be fixed whole in alcoholic solution of mercuric chloride. They are best stained with hæmatoxylin and should be counter-stained with eosin; or iron-hæmatoxylin may be used. In sections prepared in this manner, the further stages of the reproductive process may be observed. In both Gregarines numerous little nuclei, which are the ultimate products of repeated division, wander to the periphery of the parent, where they each surround themselves with a layer of denser plasma. At the same time, the principal part of the plasmic body of both parents becomes markedly alveolar and undergoes obvious degeneration. In a later stage, the little daughter-cells become completely separated from the central plasmic body. These are the gametes, the slightly dissimilar offspring of the two parent Gregarines which originally combined to form a common cyst. The gametes of the one parent are a little larger than those of the other, and they contain a somewhat larger nucleus, which is, however, not quite so rich in chromatic contents as that of the smaller gametes. These gametes then fuse in pairs; and—as the process commences with the fusion of the plasmic substance, the nuclei remaining separate for a time—it is easy to see, by the difference in size of the two nuclei, that copulation always takes place between two dissimilar gametes, the offspring in the first instance of different parents (fig. 22).

In the Gregarines of the earthworm, the difference between the two gamete forms is so slight that it requires the most careful observation to distinguish them. Thus in one species, the smaller gametes measure 3·5 μ in width and 4 μ in length, their nuclei having a diameter of 1·5 μ, while the measurements of the larger gametes are 4·5 μ, 5 μ, and 2 μ respectively. In certain polycystic varieties the sex dimorphism is much more pronounced.

After the fusion of the gametes, the copula assumes a spindle form and secretes an envelope, and in this stage it is known as the pseudo-navicella. Within the pseudo-navicella eight sporozoites are formed, the result of repeated nuclear division, accompanied by division of the cell (sporogony). These lie in the long diameter of the pseudo-navicella and are arranged in such a way that their nuclei are all congregated in the bulging central portion of the spindle. At a first glance, the sporozoites appear also to be long and spindle-shaped and to have the nucleus in the centre of the body. But it will be found

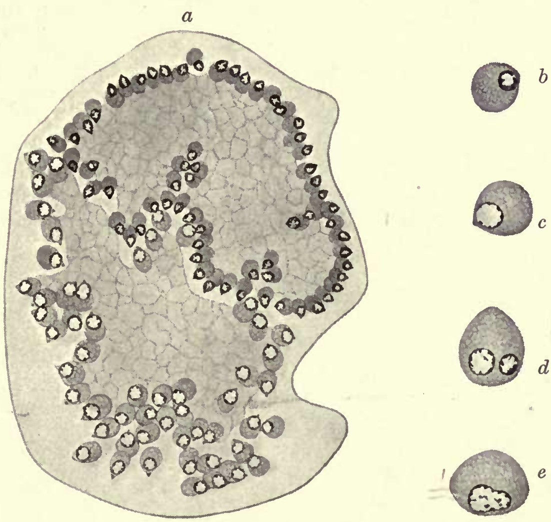

FIG. 22.—*Monocystis* from the seminal vesicles of the earthworm. *a*, Cysts, during the formation of gametes (gamogony). *b*, Microgamete. *c*, Macrogamete. *d*, Copula, before the fusion of the nuclei. *e*, Copula, during fusion of the nuclei. (After Brazil.) Magnified, *a*, 600 : 1; *b-d*, 1,200 : 1.

upon closer investigation that the nucleus is always placed at one pole of the sporozoite, and that all the blunt nucleated poles occupy the centre of the pseudo-navicella, while the pointed anterior poles run off into its two ends.

The pseudo-navicellæ vary in size in earthworm Gregarines of different species, their length varying from 0·015 mm. to 0·028 mm.

(*b*) *Gregarines of the Mealworm.*

Of the polycystic Gregarines, those most readily obtainable are the varieties parasitic in the larvæ of *Tenebrio molitor*, the mealworm, largely cultivated as food for birds. Four such varieties are recognized and, in spite of their similarity and the fact that they are found

side by side in the same intestine, they are readily distinguishable from one another.

(1) *Gregarina polymorpha* (Hammerschm.), Stein.—Cylindrical; without constriction at the junction of proto- and deutomerite; round nucleus; oval cysts; size, up to 0·35 mm. in length and 0·1 mm. in breadth.

(2) *G. cuneator*, Stein.—Of about the same size as *G. polymorpha;* constricted at the junction of the proto- and deutomerite; the protomerite is thickened at its rounded anterior end and thinned into a kind of neck where it joins the deutomerite, while the latter is thickened at its posterior end into a blunt cone; nucleus round; cysts oval.

(3) *G. steini*, Berndt.—Much rarer than the first two varieties; in size, only up to 0·15 mm. in length, and 0·04 mm. in breadth; spindle-shaped, tapering into a long cone at the hinder end; nucleus oval; cysts oval.

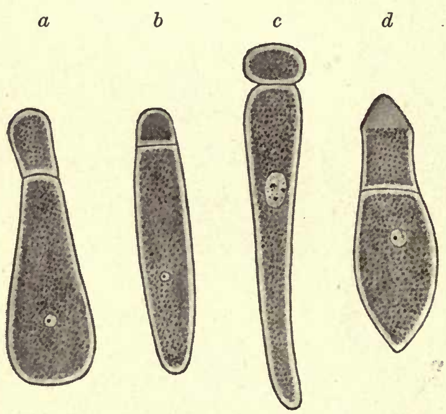

FIG. 23.—Gregarines from the gut of the mealworm. (After Berndt.) *a*, *Gregarina cuneata*. *b*, *Gregarina polymorpha*. *c*, *Gregarina steini*. *d*, *Steinina ovalis*. Magnified, *a* and *b*, 100 : 1; *c* and *d*, 330 : 1.

(4) *Steinina ovalis* (Stein), Lég. and Dub.—The smallest of the four varieties, and for this reason the one most frequently overlooked; it never exceeds about 0·1 mm. in length and 0·045 mm. in breadth; the deutomerite is broad and egg-shaped; the protomerite is short and cylindrical, ending in a more or less sharp point; nucleus round and comparatively large; cysts round or oval, about 0·1 mm. in diameter and without sporoducts.

All four of these varieties are very fairly frequent, and one of them is certain to be found after examining two or three mealworms.

The intestine of the mealworm is prepared and teazed out in the same way as the intestine of the centipede. The fat-body of the mealworm is, however, very much more developed than that of the centipede and care must be exercised to remove every particle of fat before teazing out the intestine, or the specimen will be cloudy. The Gregarines, which are usually present in large numbers, are easily seen with a low-power lens. Their movements rarely take the form of contractions; they are, rather, a species of gliding movement, which is performed, however, without any perceptible change of form.

To follow these movements, a small quantity of sepia should be added to the fluid in which the specimen is teazed out; or the Gregarines may be examined in normal saline solution in, which either Indian ink or carmine has been rubbed down until a black or deep red colour is obtained. As they move forward, the Gregarines will then be seen to leave a light track which shows up clearly in the darker medium. With a strong lens, in the interior of this light track long rows of colour granules may be seen, which appear to be clinging to the surface of a fine hyaline thread. These strings of granules sometimes appear to unite to form bundles.

This phenomenon is explained by the fact that, when in motion, the Gregarines secrete thin homogeneous threads of a sticky gelatinous substance, which cling together at the hinder end of the organism to form a sort of stalk. The refractive index of these threads corresponds so completely to that of the normal saline solution that, until the colour grains are added, they are not directly distinguishable from it. They may, however, be rendered directly visible in the following manner: A carmine preparation containing Gregarines is put into a damp chamber for about two hours. The gelatinous stalks of the moving Gregarines will then become so long that they may be perceived with a low-power lens or even with the naked eye. All the grains of carmine, with the exception of those clinging to the threads, are now removed by carefully washing out the preparation with clean normal saline. This is done by adding fresh liquid at one edge of the glass while the coloured fluid is drawn off from the opposite edge by means of filter paper. By this means, the stalks become isolated and may now be stained with methyl-violet, though they should first be fixed in alcoholic solution of mercuric chloride In changing the liquid, the flow should correspond as far as possible with the direction in which the Gregarines are moving, as their delicate stalks may otherwise be damaged.

The structure of the living Gregarine should now be carefully studied. The cuticle will be found to be thicker, and the hyaline ectoplasm thinner, than in the monocystic Gregarines of the earthworm. There is the same fine, longitudinal striation of the cuticle; and circular muscle-fibrillæ, arranged somewhat further apart, are also present between the ecto- and the endoplasm. With a high-power lens, a light homogeneous layer becomes visible between the cuticle and the ectoplasm, which is subject to considerable variation in thickness and is usually most clearly discernible during the forward gliding movements. This is the slime-secreting layer, and from it, during movement, gelatinous secretion is conveyed by means of longitudinal slits in the cuticle. The endoplasm contains a varying proportion of round granules of high refractivity. In the larger

Gregarines, these granules are so numerous as to render the organism quite opaque, the nucleus showing only as a lighter spot. In the small varieties, on the other hand, the granular contents are frequently so insignificant that the transparency of the protoplasm is unaffected by them. In the latter case, however, the endoplasm is readily distinguished from the hyaline ectoplasm by its coarsely-meshed structure. The endoplasm is not homogeneous, the body of the Gregarine being divided into two parts, placed one behind the other, and this division is frequently apparent in the external structure. The anterior portion, or protomerite, is considerably shorter than the posterior portion, or deutomerite, which contains the bladder-shaped nucleus. The two portions are separated by a divisional wall, which cuts through the endoplasm and is formed entirely from the superficial layer of the ectoplasm, with which it is continuous.

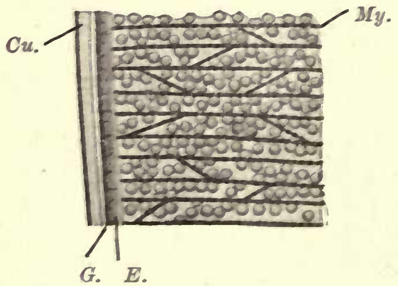

FIG. 24.—*Gregarini munieri*, Schneid., from the gut of a leaf-beetle (*Chrysomela hæmoptera*). Portion of the surface. *Cu.*, cuticle. *E.*, Ectoplasm. *G.*, Slime-layer. *My.*, Myonemes. Magnified, 1,500 ; 1. (After Schewiakoff, from Braun.)

Gregarines are frequently seen joined together in pairs, the hinder end of one being attached to the anterior end of the other. More rarely groups are formed, several (two to three, very occasionally four to five) small individuals being attached side by side to a larger one.

It is rarely possible to follow directly the process of encystment. Two Gregarines, which have become attached in the manner just described, proceed to perform rotatory spiral movements round one another, at the same time secreting a slimy envelope. As in the monocysts, the two parent Gregarines are seen lying rolled up in the young cyst. The young cysts inhabit the hinder end of the gut of the mealworm and are not very frequently encountered, though when found they are usually present in large numbers.

The further development of the cysts takes place, not in the intestine of the host, but in the open. The cysts should be isolated under the microscope and put into a damp chamber. This procedure complicates the technique, as moulds are very likely to form. Such a contingency is only to be avoided by the exercise of scrupulous cleanliness, the maintenance of control specimens, and the immediate removal of commencing growths. The developmental phenomena closely resemble those of the monocysts. A sex dimorphism in the gamete stages of *Gregarina* has not been observed. The cysts of this species differ, however, in possessing canal-like depressions in

the cyst-wall. These are the sporoducts through which, in the ripe cyst, the spores are evacuated, the canals becoming everted in the process.

In place of the Gregarines of the mealworm, the following varieties may be used for observation purposes: *G. blattarum*, which is very similar and is found with great frequency in the common cockroach; or *G. ovata*, which is egg-like in shape and compact in structure, and is found in the earwig, *Forficula auricularia*. In these varieties the epimerite, which is placed in front of the protomerite and by means of which the young Gregarines fix themselves in the intestinal wall of their host, is button-shaped and only slightly developed. Interesting modifications in the structure of the epimerite are seen in the varieties of Gregarine which inhabit the intestinal tracts of *Lithobius* and of the larvæ of *Agrion*. Those found in the *Agrion* larva are particularly well worth studying. These larvæ are prevalent in our fresh-water spaces; they are easily recognized by their slender form and by the possession of three free, leaf-shaped tail-gills.

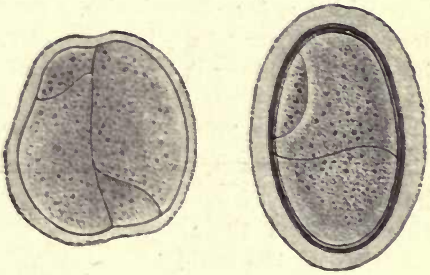

FIG. 25.—*Gregarina blattarum* in the process of encysting. (After Bütschli, from Braun.)

The study of fresh material should be supplemented by the preparation of cover-glass specimens and of sections. In transverse sections of the adult parasite, the cuticle will appear serrated at the edge. This is due to the longitudinal striation, so eminently characterestic of the order. The circular muscle-fibrillæ are seen in superficial longitudinal sections. Sections from the whole intestine are useful in identifying species; they also show the manner in which the Gregarines attach themselves to the intestinal wall of their host.[1]

Note: *Aggregata*.

The Aggregata are a class of the Telosporidia. They resemble the Coccidia in possessing no cuticle and no definite separation of ecto- from endoplasm, but in all other ways they approximate very

[1] A more detailed description of the cytology of the Gregarines of the mealworm is given by S. Kuschakewitsch, "Beobachtungen über vegetative, degenerative u. germinative Vorgänge bei den Gregarinen des Mehlwurmdarmes," *Arch. f Protistenkunde*, suppl. i, 1907, pp. 202-249.

closely to the Gregarines. They differ from both Coccidia and Gregarines, however, in that the alternation of generation, which is an essential feature in the life-history of all three orders, is, in the case of the Aggregata, associated with a change of host (from the cuttle-fish to the short-tailed crab). The relationship between the stages found in the two different hosts has only recently been established. The parasites of the crab were formerly known as Aggregata and were classed with the Gregarines, but the stages found in the cuttle-fish were mistaken for Coccidia and received the name of *Eucoccidium* (or *Benedenia*). Later authorities have preferred to class the Aggregata with the Gregarines, but in view of their dissimilar life-history it is doubtful whether this method of classification is justified.

Crabs (*Carcinas mœnas, Portunus depurator, P. corrugatus, Pinnotheres pisum, Eupagurus prideauxi*, &c.) become infected by the ingestion of cysts which have been excreted by cuttle-fish, and which release their sporozites under the influence of the gastric secretion of the crab. The sporozoites then penetrate the intestinal wall and come to rest in the sub-epithelial layer, where they grow rapidly larger. They form wart-like swellings upon the outer layer of the gut, similar to those produced by the malaria parasite in the gut of the mosquito. Their subsequent multiplication also somewhat resembles that of the malaria parasite. Numberless daughter-nuclei are formed by repeated mitotic division of the nucleus, and, at the same time, the plasm splits up into several largish portions. The daughter-nuclei move to the surface of these plasmic bodies, and there surround themselves with a small quantity of the plasma. They are now "new cells" or merozoites, and, like the sporozoites of the malaria parasite, they release themselves by violent longitudinal jerks from the plasmic masses, which remain as residual bodies.

The further development of these merozoites takes place within the gut of the cuttle-fish (*Octopus* or *Sepia*), by which the crab harbouring the parasites is devoured. Here, also, the merozoites bore their way into the intestinal wall, where they usually come to rest in a cell of the submucosa, and where they develop into the large immotile gametocytes. The female gametocyte[1] is richer in reserve material than the male. It is also, as a rule, much larger, the diameter in *Aggregata legeri*, from *Octopus*, measuring 0·25 to 0·3 mm. The nucleus is strikingly large and may measure as much as 0·1 mm. in diameter.

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[1] As the outcome of Siedlecki's experiments they have always, up till now, been regarded as the macrogametes of the so-called *Eucoccidium*. The organism was believed to encyst, the presence of a membrane-like attachment from the host-cell lending support to the supposition.

The chromatic contents of this nucleus now become very much reduced. The nucleus breaks up and the first spindle is formed from a minute quantity of the original chromatin. Numerous daughter-nuclei are formed, the result of repeated mitotic division, and these distribute themselves over the periphery of the parasite. The entire organism becomes irregularly wavy in form, owing to the formation of deep folds in its surface. Each nucleus surrounds itself with a portion of the parent plasma, so that, finally, the entire organism splits up into numerous pear-shaped female gametes which, formerly, were regarded as sporoblasts. There is no residual body. The nuclear changes differ slightly in the male gametocyte. They result in the formation of numerous microgametes, which carry two free flagella at their anterior end. These microgametes are long and narrow in shape, those of *Aggregata spinosa* measuring 1 mm. in length, while they rarely exceed 0·003 to 0·005 mm. in thickness. Fertilization has not been directly observed. It is certain, however, that the process of fertilization is followed by the encysting of the copula, which then divides within the cyst into a varying number of sporozoites (three to four in the varieties parasitic in *Sepia*, eight to twenty-four in those found in *Octopus*). The ripe cysts containing spores are passed out of the body of the octopus in the fæces, and the sporozoites are not released until the cysts reach the intestine of a suitable species of crab. The older notion that the spores were released within the gut of the octopus has proved erroneous.[1]

The Aggregata are, for many reasons, a particularly interesting group. They illustrate very clearly the necessity for extreme caution in tracing out the developmental history of Protozoans. The descriptions of the so-called *Eucoccidium*, which are to be found in all text-books, are absolutely misleading because they are founded upon insufficient and incorrect data. In spite of the care with which Siedlecki conducted his investigations, they are yet incomplete in two essential points. He was unable to observe directly either the process of fertilization or the release of the sporozoite, and the gaps were filled from the life-history of the Coccidia, with which class the organism was at that time thought to have points of resemblance.

Fresh material and cover-glass specimens should be prepared, and sections in series will also be found very instructive.

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[1] Th. Moroff, "Die bei den Cephalopoden vorkommenden Aggregata-Arten als Grundlage einer kritischen Studie über die Physiologie des Zellkerns," *Arch. f. Protistenkunde*, vol. xi, 1908, pp. 1-224; L. Léger et O. Dubosq, "L'Evolution schizogonique de l'Aggrégata (Eucoccidium) eberthi (Labbé)," *ibid.*, vol. xii, 1908, pp. 44-108.

Class V. **Ciliata.**

The "Filamented Infusoria," or Ciliates, are so called on account of the peculiar nature of their organelles of movement. They are to be distinguished from the sub-class Suctoria, which have no practical interest and will not be described here. The motor appendages, or "cilia," are present in very large numbers in the Ciliates, and the method of their arrangement is used as a basis for classification. In addition to their locomotor function, they serve also as organelles of nutrition and, like the whips of the Flagellates, they are attached to the ectoplasm by means of basal structures. The superficial layer of the ectoplasm is hardened to form a definite cortex or pellicle, which assures to the parasite constancy of form. As a rule, food is ingested by the agency of a special oral part (cytostome), from which a canal of varying length (cytopharynx) leads into the endoplasm. A special anal part (cytopyge) is frequently present, by means of which undigested food remnants are excreted by the organism. As a rule, one or more contractile vacuoles are present, and, unlike the other classes of the Protozoa, they are present in both the parasitic and non-parasitic varieties. The number and position of these contractile vacuoles varies in different species, but never in individuals of the same species. The Infusoria, both Ciliates and Suctoria, are characterized by the possession of two nuclei, which differ in shape and in function. The larger is known as the macronucleus, and the smaller as the micronucleus. The latter is the sex nucleus, and plays an important part in the process of reproduction (see text-books of Zoology). Only one nucleus of each kind is present, as a rule, though this is not invariable. The Opalina are the only members of the Ciliate class which do not present this nuclear dimorphism. The Ciliates almost invariably multiply by simple binary fission, though *Ichthyophthirius* (see p. 91) is an exception to this rule. The organism encysts as a protective measure in case of dryness or other unfavourable external condition, and it is probable that, in the case of the parasitic varieties, infection is conveyed at this stage of their development.

The Ciliates vary very much in general form. To obtain an adequate idea of their extreme diversity, the student should extend his observations to the non-parasitic varieties. Free-living Ciliates are to be found in every pond, and abundant material may be obtained by pouring water over a little fresh hay and allowing the infusion to stand for one to three weeks. The extreme motility of the organisms may be reduced by adding a small quantity of carragheen to the water, when they may be examined singly with the aid of a strong lens. Both the arrangement and the movements of the cilia should be observed, as well as the regular pulsation of the contractile

vacuoles. The manner in which nutriment is taken up may be seen by rubbing down a little powdered carmine in water and adding it to the medium in which the organisms are examined, which should not, in this instance, be thickened with carragheen. A good deal is to be learnt from the living organism when stained with neutral red. The arrangement of the cilia is an important factor in determining species.[1]

The Infusoria are best fixed with alcoholic solution of mercuric chloride or with osmium vapour. In the latter case the material is placed upon a cover-glass, which is held inverted over a vessel containing osmic acid until the parasites turn brown. The cilia may now be coloured with Schuberg's modification of Löffler's flagella stain. The objects are immersed in a mordant mixture, consisting of 10 c.cm. of a solution of 20 gr. tannin in 80 c.cm. distilled water, 5 c.cm. of a saturated solution of ammonio-sulphate of protoxide of iron, and 1 c.cm. of a watery or alcoholic solution of wool-black. They are put into a damp chamber for half an hour to one hour, and are afterwards washed in water and absolute alcohol. They are stained (half an hour to one hour) in a concentrated solution of fuchsin in aniline water, to which a $\frac{1}{10}$ per cent. solution of soda has been added in a quantity sufficient to start precipitation. Specimens must be cleared in xylol, as oil of cloves will spoil the colour. They should on no account be dried by the methods employed for bacteria; the entire process must be carried out wet, the different liquids being changed by means of filter paper introduced between the cover-glass and the slide.[2]

(*a*) *Infusoria found in the Intestine of the Frog.*

Some of the most interesting of the parasitic Infusoria inhabit the large intestine of the frog. The abdominal cavity of a freshly-killed frog should be opened, and the short, thick, terminal portion of the intestinal canal should be freed and cut open. A small quantity of its greenish contents is removed and diluted with a little normal saline. As a rule, whitish, actively motile dots may be seen macroscopically, and these will be most numerous upon that surface of the intestinal contents which has been in contact with the intestinal wall. These dots are Opalina. They are flat, disc-like Protozoa, and their entire surface is covered with cilia, the movements of which resemble the

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[1] F. Blochmann, "Die microscopische Tierwelt des Süsswassers," part i, 2nd ed., Hamburg, 1895.

[2] A. Schuberg, "Ueber Cilien und Trichocysten einiger Infusorien," *Arch. f. Protistenkunde*, vol. vi, 1905, part i, pp. 47-60.

waves which pass over a field of corn. These movements should be carefully studied, carragheen (which has been previously swelled in normal saline) being added wherever necessary, though as a general rule the bowel-contents will be sufficiently thick without it. The cilia are arranged in longitudinal rows, corresponding to a fine longitudinal striation of the surface of the parasite. There is no cytostome, nutrition taking place by endosmosis. There are also no contractile vacuoles. The Opalina resemble the true Infusoria in possessing a ciliated exterior, but they differ from them in the method of their reproduction[1] and in the absence of anything resembling nuclear dimorphism. Three varieties of Opalina are found in indigenous frogs. They are as follows :—

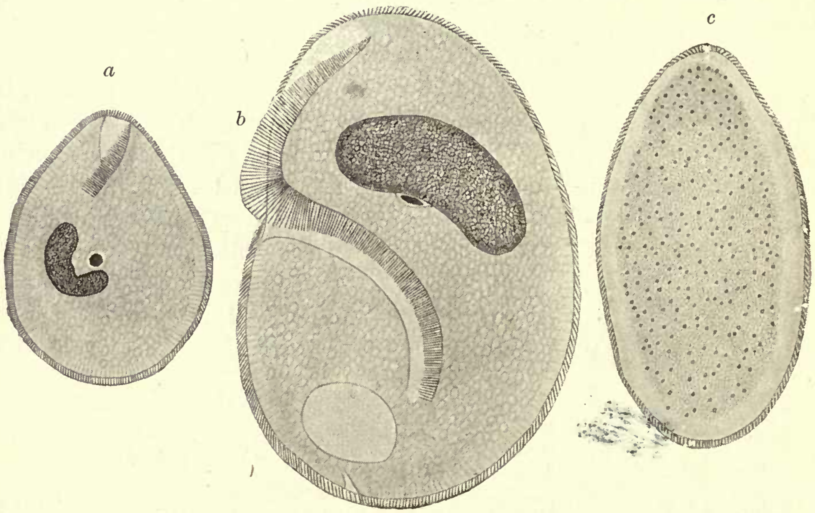

FIG. 26.—Infusoria from the large intestine of *Rana temporaria* (original). *a*, *Balantidium entozoon*. *b*, *Nyctotherus cordiformis*. *c*, *Opalina ranarum*. Magnified, *a* and *b*, 375 : 1 ; *c*, 125 : 1.

Opalina ranarum (in *Rana temporaria*).—Much flattened in form ; this parasite is about half as broad as it is long, the greatest breadth being at, or just behind, the middle line.

O. dimidiata (in *R. esculenta*).—Spindle-shaped : about four times as long as it is broad, the greatest breadth being anterior to the middle line.

O. zelleri (also in *R. esculenta*).—Very clumsy in shape ; at least half as broad as it is long ; not nearly so much flattened as

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[1] E. Neresheimer, "Die Fortpflanzung der Opalinen," *Arch. f. Protistenkunde*, suppl. i, 1907, pp. 1-42.

O. ranarum, being more like a barrel in shape. It is much rarer than the other two. All three varieties are furnished with numerous similar, small, round nuclei (fig. 26, *c*).

Although the Opalina are of most frequent occurrence, two other much smaller Infusoria are sometimes met with in the intestine of *R. temporaria* and *R. esculenta*. They are *Balantidium entozoon* and *Nyctotherus cordiformis* (fig. 26, *a* and *b*), which belong to the order Heterotricha, and are closely related to the intestinal Infusoria of man. This order is characterized by the possession of a uniform covering of similar cilia and by an "adoral zone," a row of larger cilia or membranelles, which runs along the peristome from the anterior extremity of the parasite to its oral opening. In *Nyctotherus* the mouth continues as a long cytopharynx, and the adoral zone is prolonged to its end. The arrangement and the movements of the cilia should be noted, as well as the action of the contractile vacuoles, of which *Nyctotherus* possesses one, while four are present in *B. entozoon* (not represented in fig. 26, *a*). In *Nyctotherus* there is also a short anal tube, situated at the posterior end, close to the contractile vacuole. The macronucleus in *Nyctotherus* is bean-shaped and the micronucleus lies close to its hinder concave surface. In *Balantidium*, it is round, and is, as a rule, easier of detection, being situated within the curve of the macronucleus, which is very much bent, varying in shape from that of a kidney to that of a horse-shoe.

Cover-glass preparations of the whole organism are fixed in alcoholic solution of mercuric chloride and stained with hæmatoxylin, counter-staining with eosin. The finer details, such as the insertion of the cilia and the structure of the nucleus, are only to be seen in sections, which should be stained with iron-hæmatoxylin.

(*b*) *Balantidium coli.*

Opportunities for the study of the parasitic Infusoria of man are rare in this country. *B. coli*, the best known of these and, on account of its pathogenic significance, the most important, is principally found in the north-east of Europe. Occasionally, though this is rare, fresh material may be obtained in East Prussia. There is a *Balantidium*, however, which is invariably present in the rectum of swine and which is so similar to the parasite found in man, that the two organisms have, up to now, been considered identical.

The rectum should be tied at both ends before removal and it should be taken from the carcase immediately after slaughter. It should be wrapped in a thick cloth to prevent cooling during transit from the slaughter-house to the laboratory, and the examination should be made as soon as possible—certainly upon the same day.

Where this is impossible, the portion of intestine should be kept in a thermostat at a temperature of 37° to 38° C.

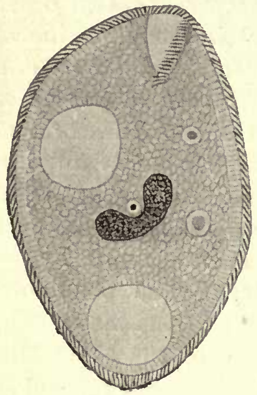

Fig. 27. — *Balantidium coli*, from an ulcer of the large intestine of man. Magnified, 600:1. (Original.)

It is very rare to find the intestine of swine free from *Balantidia*. The living organism is best seen by diluting a small quantity of the bowel-contents with normal saline solution, which has been previously heated to 37° C. The Infusoria may be seen with a low-power lens and are readily distinguished by their movements. The structural details are only to be seen with a strong glass. The parasite differs somewhat from *B. entozoon;* the peristome is shorter, the individual cilia are shorter, and there are only two contractile vacuoles (fig. 27). If the material is allowed to cool, or if the portion of intestine is kept for a time before examination, round cysts of *Balantidia* will be found, while the free forms will be proportionately fewer. Occasionally it is possible to follow the actual process of encystment under the microscope.

(c) *Infusoria found in the Stomach of Ruminants and in the Cæcum of the Horse.*

A large and interesting variety of Infusorians is found in the rumen and reticulum of ruminants. The stomach of a freshly killed animal should be punctured, and the fluid which flows from the perforation (it should be fairly free from food-remnants) is caught in a glass-tube. This should be wrapped in a cloth and carried in the pocket to the laboratory. If kept in a thermostat at a temperature of 35° to 36° C. the Infusoria will live, certainly for one day, but never longer than three, owing to fermentation of the stomach-contents. The objects must be examined upon a warm table, as the greater number of the Infusoria found in the stomach of ruminants possess a retractive peristome, which is only released under the influence of a certain degree of warmth. A temperature of about 30° C. is sufficient, and this will also slightly reduce the extreme motility of the organisms. For preservation, a small quantity of the material is put into a warm glass and hot alcoholic solution of mercuric chloride is poured over it.

The Infusoria which live in the gastric secretion belong to a large number of different species, and are sometimes distinguished by their bizarre form. They frequently put out stiff, thorn-like prolongations,

which vary in number and arrangement.[1] For further details the student is referred to the special literature on the subject.

The cæcum of the horse is also the habitat of a large variety of Infusoria, which, in many cases, resemble those described above. To obtain the Infusoria in a medium of suitable fluidity, the bowel-contents should be filtered through a warm cloth. The parasites are, however, more sensitive than those found in the stomach of ruminants and, even with the aid of a thermostat, it is not possible to keep them alive for longer than two or three hours. The species most frequently met with, which is also the largest, is *Cycloposthium palmatus.* It is characterized by a remarkable double organ of movement; a bunch of six strong cilia, which proceed from a short tube-like sheath, is placed upon either side at the hinder end of the body. Other varieties, together with their more intimate structural details, are described by Bundle.[2]

(*d*) *Ectoparasitic Infusoria of Fish.*

Infusoria are frequently found upon the skin and gills of fresh-water fish, and in fishes and aquaria these may have a pathogenic significance. The most interesting varieties and the ones most frequently met with are *Cyclochæta domerguei,* Wall., a semicircular Infusorian of the order Peritrichida, found upon the gills, to which it attaches itself by means of its modified and sucker-like peristome; and *Ichthyophthirius multifilius,* Fouqu., a nearly round and almost opaque Holotrichian, which forms small white pustules under the epidermis. It encysts after the bursting of the pustules and the cysts fall to the bottom of the water, where numerous daughter-cells are formed within the cyst. Both of these parasites may be fixed in cover-glass specimens by the method described for *Costia* (see p. 41).

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[1] A. Schuberg, "Die Protozoen des Wiederkäuermagens," *Zool. Jahrb. Abt. f. Syst.*, vol. iii, 1888, pp. 364-418; A. Schuberg, "Ueber einige Organisationsverhältnisse der Infusorien des Weiderkäuermagens," *Sitzber. d. phys-med. Ges Würzburg*, November 21, 1891; R. Eberlein, "Ueber die im Wiederkäuermagen vorkommenden ciliaten Infusorien," *Zeitschr. f. wiss. Zool.*, vol. lix, 1895, pp. 232-304.

[2] A. Bundle, "Ciliate Infusorien in Cæcum des Pferdes," *Zeitsch. f. wiss. Zool.*, vol. lx, 1895.

PART II.

HELMINTHES.

The term Helminthes (intestinal worms) includes all the parasitic worms. These, though belonging to widely different zoological classes, yet possess striking similarities of structure and development, the result of modifications brought about by the conditions under which they live. To this group belong the Trematodes (sucking-worms), the Cestodes (tapeworms), Nematodes (threadworms), and the Acanthocephales (hooked-worms). Formerly it also included the Cysticerci (bladder-worms) and the Linguatulidæ (tongueworms), but the former are now recognized as a developmental stage of the Cestodes, while the latter are modified Arthropods. The Hirudinea (leeches), which live more in a state of brigandage and attack both man and the lower animals, are certainly parasitic in their mode of life, though they are not usually classed as Helminthes. The group, moreover, does not include other parasitic Metazoa, whether occasional or permanent in their attachment; these should be classified according to their distinguishing characteristics.

The majority of the intestinal worms live as endoparasites in the interior of the body of their host. Their usual habitat is the bowel and its appendages, though certain varieties, such as the monogenetic Trematodes, are to be found upon the surface of the body, in hollow organs which are readily accessible from the exterior (oral cavity, urinary bladder). The Trematodes, Cestodes and Acanthocephales are entirely parasitic in their manner of life. Free-living stages occur among the Cestodes, and, in certain Nematodes (Angiostomides), free-living and parasitic generations alternate. There are also true free-living Nematodes which may occasionally live as parasites (facultative parasitism), while a large number of Nematodes never become parasitic at all. These are described as "free-living Helminthes."

The number of the varieties observed in man has largely increased in later years. In all, eighty-three species are recognized; of these sixteen are Trematodes, twenty Cestodes, forty-five Nematodes, and two Acanthocephales. A certain number only are specific, *e.g.*,

confined exclusively, or almost exclusively, to man. The others are normally parasitic in the domestic or wild animals; they may, however, adopt man as a host, in which case they are described as "occasional" parasites. In many cases, the normal hosts of parasites which are occasional in man have not as yet been discovered.

It is not possible, nor is it very necessary, to examine all these many varieties of Helminthes, some of which are exceedingly rare. It will suffice for the student to recognize from personal observation those species which are of frequent occurrence; while the rarer sorts, should they come under his notice, may be identified by the aid of the text-books of the subject.

CHAPTER I.

DIRECTIONS FOR OBTAINING MATERIAL.

It is probable that every vertebrate species may, under conditions, harbour Helminthes. Naturally, they are not to be found in every individual, nor will all the parasitic varieties to which it is subject be found in a single member of the host-species. Certain host-species are less frequently attacked than others, while the percentage of affected individuals of the same species is dependent upon locality and age. In other words, it is dependent upon the food, the nature of which changes at different stages of growth. As long as food is taken in a form which precludes the transmission of Helminthes, for so long will the individual be preserved from infection—at least, from infection by food. As a general rule, the sucking young of mammals do not harbour Helminthes, although infection with certain varieties may occur as a result of external conditions; or the young mammal may inadvertently swallow some small animal containing parasites. But, speaking generally, material for demonstration purposes should be sought in animals which have passed the stage of early youth.[1]

We will now assume that a cat is to be examined for Helminthes. We know, from the earlier text-books, that the cat harbours full-grown worms, as well as certain of their developmental stages. The former inhabit the intestine, the gall-bladder, the gall-ducts, and, occasionally, the urinary bladder; while the latter are found encysted on and in different internal organs (bowel-wall, liver, muscular structure), or free in the great body-cavities. The frequency of occurrence varies with the species. Some varieties are found in every individual; such are

[1] O. von Linstow's "Compendium der Helminthologie" (Hanover, 1878, 1889) will be found very useful for reference. Although out of date in certain particulars, it is, as far as it goes, an exceedingly reliable work.

Ascaris mystax (now called *A. canis*) and *Tænia elliptica* (*Dipylidium caninum*). Other varieties, though comparatively frequent, are by no means invariable; such is *T. crassicollis*. Others again, such as *Bothriocephalus felis*, *Opisthorchis felineus* (v. Linstow calls it *Distomum lanceolatum*), are comparatively rare; while certain imported varieties, as *Oxyuris compar*, may be occasionally met with. There are also a small number of quite rare species, and others again which are rare in the cat, though encountered frequently in other hosts.

The animal should be chloroformed, and examined as soon as possible after death.[1] It is laid upon its back and fastened securely by the legs, which should be turned outwards. The neck should be drawn back, the head fixed, and the skin freed from any ectoparasites (fleas, lice, ticks) which may be present. An incision is now made in the skin down the entire length of the body, beginning at the chin and ending at the anus. The skin is rapidly freed from the sides, the rib-cartilages are severed, and the breast-bone removed. The peritoneal cavity is opened up by means of a long incision through the thin muscular wall of the abdomen. As *Cysticercus elongatus*, Blbrg., is occasionally found in both the peritoneal and the pleural cavities; this should be first sought for, the viscera being raised in such a way as to leave the cavities free to inspection. The lungs should next be examined, and here a roundworm, *Strongylus nanus*, A. Müll., which varies in length from 5 mm. to 10 mm., may be found. Its presence is betrayed by the condition of the lung, and may be verified by microscopic examination of the bronchial mucus, oval eggs in all stages of development and free embryos being found. In such a case, the bronchi should be opened down to the smallest branches, and the worms removed from the lung-tissue.[2] The bowel should next be examined. After removing the omentum, the small intestine should be cut across immediately behind the stomach. The cut end should be held with the left hand (or by means of tweezers), and the mesentery separated with a scalpel from the whole of the small intestine, as far as the commencement of the colon. The small intestine is next cut across at the cæcum; it is laid upon a

[1] Wherever possible, Helminthes should be sought in the freshly killed cadaver, as many species do not long survive the death of their host. The decomposition of Helminthes follows very quickly after death. The Tæniæ of birds, for instance, possess but slight power of resistance. Nematodes, on the other hand, are comparatively robust, as are the encysted forms of the greater number of Helminthes. The latter are frequently found alive in organs which are already in a state of maceration.

[2] In North America, *Paragonimus westermani* is found in the lung of cats. This parasite may occur in man, but its normal host is the tiger. It occurs also in swine and dogs, but only when their habitat is extra-European.

board and split lengthwise with scissors with protected points. This manipulation should be performed with great care, in order that any Helminthes present in the bowel may not be damaged. The blunt end of the scissors should be inserted into the lumen of the bowel, and it should be kept quite close to the bowel-wall, of which only a small portion should be cut at a time. When split along its entire length, the bowel is opened out, fixed with pins to the board, and its contents examined. Helminthes of sufficient size to be perceived with the naked eye will have been seen during the process of cutting, and their site of attachment should now be noted. Many species favour special sections of the small intestine, just as others are only to be found in the stomach or the cæcum Regular distribution of the same species through the entire length of the small bowel is very rare in a freshly killed host.

Position, method of attachment, and movements of the Helminthes should be noted and the free individuals should be lifted out with the spatula and put into shallow vessels containing normal saline solution. The Helminthes which are not free should next be detached, wherever possible, from the bowel-wall. This can generally be accomplished by digging into the mucosa at the point of attachment, and bringing a portion away on the spatula with the worm. The method is not always successful, however. Many Cestodes (though this does not apply to those of the cat) as well as Echinorhynchus varieties, strike so deeply into the mucosa that they penetrate the muscular structure of the bowel-wall. Liberation, in these cases, is much more difficult and is frequently to be effected only by special preparation under the microscope. It is not advisable, in any case, to release all the attached individuals. In certain cases, the portion of the bowel-wall which has become changed by the agency of the parasite should be cut away together with the scolex, and the two fixed whole together. This should be carried out as expeditiously as possible, as the worms frequently release their hold when disturbed or as the bowel cools, and it is rare to find them attached except in a quite fresh organ.

The Helminthes most frequently encountered in the intestine of the cat are *Ascaris canis* (=*A. mystax*) and *Dipilidium caninum* (=*Tænia elliptica* =*T. cucumerina*). Both varieties, and especially the latter, are as a rule found in large numbers.[1] Both may occur in man and, for this reason, they have a special practical interest. Ascarides (fig. 28) live free in the lumen of the bowel; *D. caninum*, the "cucumber-worm,"

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[1] In Italy, *D. trinchesii*, Diam., and *D. pasqualei*, Diam., are found in the domestic cat, while *D. chyzeri*, v. Rátz, is found in cats in Hungary. (See V. Diamare, "Il genere Dipylidium," Naples, 1893; also v. Rátz, *Centralbl. f. Bakt., Par. und Inf.*, 1, part xxi, 1897, p. 465).

(fig. 29), is generally attached at the head end, but is easily released. Careful measures are required to obtain the worm whole, as it breaks easily at the thin anterior end. When these worms are present in large numbers, the mucosa in the region of attachment may be scraped away with a spatula and the whole complex of worms, mucosa, and bowel-contents transferred to vessels containing normal saline. After a short interval, the heads will become detached from the mucosa of their own accord.

Single proglottides should also be carefully sought. They resemble cucumber-seeds in shape and are reddish in colour. They will be

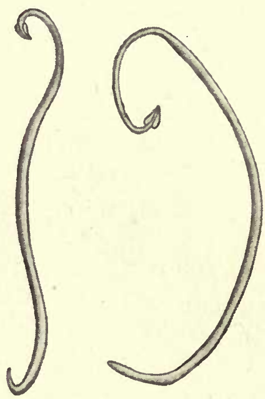

FIG. 28.—*Ascaris canis*, L. (natural size). Small variety from the cat. Left, male; right, female.

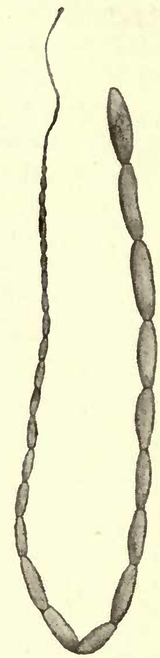

FIG. 29. — *Dipylidium caninum*, L. (natural size).

found in the neighbourhood of the worm itself, or in the lower portions of the small intestine. They may also be found in the large bowel; but, wherever they occur, they are always on their way to the exterior.

Of the larger tapeworms, *T. crassicollis*, a thick, heavy worm closely related to the *T. solium* of man, is the one most frequently encountered in the cat. In localities where Bothriocephalus occurs, a slender variety of the human *Dibothriocephalus latus* is occasionally met with, and, though more rarely, the small *Bothriocephalus felis*, Crepl.

Equally rare are the Trichocephales, which attach themselves by means of their thin anterior end to the mucosa of the cæcum, though they may occur in the lower end of the small intestine.

A sexually mature, very small roundworm, *Ollulanus tricuspis*, inhabits the gastric mucosa. Part of its brood passes out with the

fæces; other individuals wander about the body of the host and become encysted in the pleura, liver, and particularly the lungs.

Next to the intestine, the liver is the visceral organ which is most frequently attacked. Trematodes are found in the gall-ducts and gall-bladder. In North Germany, where cats are very largely infected, the varieties usually met with are *Opisthorchis felineus*, *Metorchis albidus*, and *M. truncatus;* though these also occur in other localities. In North America, *O. felineus* is replaced by a form, *O. pseudofelineus*, which closely resembles it, while *Clonorchis endemicus* is met with in Japan. V. Siebold's assertion that *Dicrocœlium lanceatum* (=*Distomum lanceolatum*) occurs in the liver of the cat has proved erroneous; the parasites which he saw were undoubtedly *O. felineus.* The true lancet-worm has, however, been observed quite recently in the cat, but its occurrence is extremely rare.

The gall-bladder should be removed whole, placed in a watch-glass of sufficient size, and then opened along its greatest length. Trematodes will be seen macroscopically in the gall as it runs out, and their presence may be demonstrated beyond a doubt if the gall is diluted with a little normal saline. It may happen that the secretion is free from parasites; the liver, however, should be examined in any case, as these species inhabit the gall-ducts, the larger branches of which should be opened. The entire liver is now cut with a scalpel into slices of about a finger's breadth in thickness; these should be lightly pressed to force the worms out of the smaller branches of the gall-ducts. They frequently emerge with a jerk on to the cut surface and should be removed with a spatula or paint-brush. The cut surface, upon which there is inevitably a certain amount of blood, is scraped with the back of the scalpel and the material is put into shallow glasses containing normal saline solution. As a certain proportion of the worms are likely to remain in the gall-ducts, the slices of liver should be put on a plate, covered with normal saline solution, and allowed to stand for a time. The worms will be found in the liquid or upon the cut surfaces, or they will emerge if the slices of liver are gently pressed.

In the case of host-species which harbour parasites in the portal vein, a similar method of examination is followed. The vessel is opened, the blood caught in shallow bowls, and the liver cut into slices. Both blood and the material obtained by scraping are spread in small quantities upon plates, one half of which has been blackened. The material should be thinned with normal saline and the fluid should be slowly moved from side to side during inspection.

In certain host-species, encysted Helminthes are met with in the liver, the most prevalent being the bladder-worm. They are sometimes attached to the surface, sometimes they lie deeper. They may

be peeled out, together with the cystic membrane, which is derived from the tissues of the host; or the membrane may be carefully cut through and the parasite taken out. Mature Nematodes and Cestodes are found in the liver of very few host-species.

The other internal organs are examined in a similar manner. Hollow organs are slit up and solid organs carefully cut into slices. Ample material is forthcoming from indigenous vertebrates, and it may be supplemented by material obtained from slaughter-houses. Fresh carcases of extra-European animals, as also of the rarer European land vertebrates, can sometimes be obtained from menageries and zoological collections, while foreign fish may be got from live-stock dealers and hatcheries. It should be borne in mind, however, that animals which have been long in captivity, or have died after lingering illness, do not as a rule yield very abundant material. As a consequence of changes in diet, or of illness, Helminthes are frequently absent from the intestine, though they are sometimes found in the other organs, generally in the encysted stages. The chances of finding parasites in the intestine are greater in the case of animals which have died soon after importation. The attendants, moreover, are aware that Helminthes are passed in the dung of freshly imported animals, and use should be made of this knowledge.

Even in fishing towns, fish are usually emptied of their viscera before coming upon the market, and for this reason the encysted forms of Helminthes are the ones most frequently found in them. Occasionally, they are found free upon the skin and gills. Interesting material is sometimes found in the gut of certain species; such are the shark and the skate.

Invertebrates, both of the land and of fresh and salt water, may also harbour Helminthes, though in certain developmental stages only. These occur free or encysted in the substance of the internal organs and, with the exception of the gut, they are rarely found in the hollow organs. In addition to the Protozoa, the parasites most frequently present in the gut are a small species of Nematode.

CHAPTER II.

METHODS OF PRESERVING HELMINTHES.

Helminthes should be examined in the first instance in the fresh state. As a general rule, they are sufficiently transparent for the purpose, and this transparency may be increased by gently pressing them between the glass slide and the cover-glass (or two glass slides may be used). It is very necessary, however, to preserve them, both for museum purposes and for section cutting. The method varies

according to the species to be examined and the purpose for which the specimen is required.

(*a*) Method of Preserving Specimens Whole.

(1) *Trematodes.*—Flat worms, of slight muscular structure, are best for preservation whole, while very large and thick round worms are unsuited to the purpose. The specimens should be washed in normal saline solution immediately after their removal from the host; they should be freed from mucus, &c., with a brush, and then stretched out upon a glass slide. A cover-glass of sufficient size and thickness should be smeared with a drop of the fixing fluid and inverted on to the worm, which, as a rule, remains stretched out on the slide, without bending or folding, and becomes flattened out. This flattening process may be lessened or increased according to the amount of fluid introduced under the cover-glass. It should be added drop by drop at one edge, while the superfluous fluid is drawn off by means of filter paper from the opposite edge. The worm should not be crushed, nor should it be flattened to such an extent as to change the shape of the organs. The expulsion of ova from the uterus is a sign that the pressure is considerable, while the passage of fæces from the oral sucker points to a still higher degree of pressure. It is a good plan to arrange supports by the side of the object to be examined, and these may be cut from writing paper, blotting paper, post-cards, or cartridge paper, in accordance with the thickness of the parasite. The strips should be quite narrow and should be laid upon the glass slide in such a way that, when the cover-glass is applied, they may be under it.

There are certain varieties, even among the smaller worms, in which muscular action is strong, and it will be found that the pressure of the cover-glass alone, even when increased by the withdrawal of the fluid, is insufficient to keep these varieties flattened out. In such cases a glass slide, or small pieces of lead or iron, should be put upon the cover-glass; or it may even be necessary to use a press. Extra pressure is indispensable when preparing large flatworms.

The best fixing fluid is undoubtedly a formula used by D. Hofer. Fifty parts of a saturated watery solution of picric acid, are mixed with 48 parts of water, and 2 parts of glacial acetic acid. The mixture acts first upon the edges of the specimen and works inwards very rapidly, but it is sometimes unable to penetrate to the two surfaces touching the glass. In that case, the unaffected parts will remain transparent while the edges will be yellow and opaque. The defect may be remedied by gently raising the cover-glass and allowing it to fall back again into place. It may, however, be necessary to add

fresh liquid, after quickly removing what is left of the original application; this manœuvre should be repeated until the entire worm becomes opaque. It must be borne in mind that the lifting of the cover-glass is very liable to tear the specimen, especially when the preparation has been subjected to pressure. Should this appear imminent, the cover-glass should be allowed to fall back into place and fresh liquid must be added at the edge of the glass until the cover-glass floats, either quite free or with the worm attached. In either case the specimen will be left exposed, either upon the glass slide or the cover-glass, and the process of fixing may be completed by adding fresh fluid. Or the worm may be freed from the glass by pouring the Hofer mixture over it; it should then be transferred to a watch-glass by means of a fine brush. If the object is fixed upon the glass slide, as soon as it becomes opaque it should be washed off into a watch-glass, and it should, in any case, be allowed to remain for a short time in the fixing fluid. The fluid is then removed by means of a pipette and clean water is added. This is quickly drawn off and replaced by 45 to 50 per cent. alcohol. The object should remain for a few minutes in the alcohol, which is then drawn off and replaced by 70 per cent. alcohol. This alcohol is changed every few hours until it ceases to contain picric acid, *i.e.*, until it no longer acquires a yellow colour. The process may occupy several days. The object is next put into 80 per cent. and afterwards into 96 per cent. alcohol, and in this stage may be kept for any length of time. Specimens should be stored in closely covered jars; or they may be put into glass tubes and closed with wadding (not cork); or into glass bottles with well-fitting glass stoppers. They should be carefully labelled, the description including the name and pedigree of the host, the organ from which the parasite was taken, method of treatment, date.

Roundworms and muscular flatworms may be prepared whole in the following manner. The worms should be cleansed and put into a watch-glass containing a small quantity of normal saline. Warm saturated watery solution, or warm alcoholic solution, of mercuric chloride should be poured over them and the objects allowed to remain in soak until they become opaque, which will be in a few minutes. If the watery solution is used they should be rinsed in normal saline, but in 70 per cent. alcohol if the alcoholic solution is used. In either case, a few drops of tincture of iodine should be added to the rinsing fluid to remove superfluous sublimate from the tissues, and the liquid should be changed until it ceases to lose its colour. Objects washed out with normal saline are treated with alcohol of gradually increasing strength and may be stored in 95 per cent. alcohol. Objects rinsed in alcohol are put straight into 80 per cent. and afterwards transferred

to 95 per cent. alcohol. For the purpose of more minute investigation, they are completely dehydrated in absolute alcohol, cleared in creosote, and examined upon hollow-ground glass slides.

Simple preservation in alcohol also provides very useful material, as exemplified by the collections of the older helminthologists. And to-day there is no better method of preserving Helminthes for museum purposes. It is a very moot point as to whether specimens preserved with the metallic salts are as enduring as those kept in spirit.

(2) *Cestodes.*—In its first stages, the process is the same as that described for Trematodes, but the details of preparation must be carried out as expeditiously as possible, owing to the fact that changes, such as the detachment of the cuticle and the hooks, occur if the specimens are left for long in a saline solution. Small varieties and small individuals of larger varieties, if not too large to be covered by a cover-glass, should be prepared whole. Larger varieties should be divided and, in the case of the giant tapeworms, the scolex and specimens of both mature and young proglottides should be preserved. As with the Trematodes, Hofer's picrin mixture may be replaced by 70 per cent. alcohol, with the addition in this case of a few drops of acetic acid to dissolve out the calcareous bodies, which are present in large quantities in most Cestodes.

(3) *Nematodes.*—The preservation of Nematodes whole is a somewhat difficult matter, owing to their highly resistant cuticle. This is liable to form folds which obscure the view of their internal organization. Nematodes are, moreover, very impermeable to staining reagents and it is useless, in any case, to attempt to stain the whole worm. Mounting in Canada balsam or other gums is also impossible, owing to the almost unavoidable shrinking of the cuticle when the specimen is cleared in creosote or turpentine. Good results may, however, be obtained by finishing in glycerine and mounting in gelatine (gelatine 20·0, glycerine 100·0, aqua dest. 120·0, acid carbol. 2·0).

Nematodes should not upon any account be left for long in saline solution; the cuticle becomes detached, the worms swell up and finally burst, allowing the viscera to protrude. For this reason they should be fixed as soon as possible after they are found. The smaller varieties should be laid in pairs, male and female, upon a glass slide and covered with a cover-glass of sufficient size. They should be fixed in weak (30 per cent.) alcohol or in Müller's mixture (potassium bichromate 2·5, aqua dest. 100, sodium sulphate 1·0), and the liquid should be introduced under the glass in a quantity sufficient to produce slight capillary pressure. The specimens should now be kept for a few days in a damp chamber, which may be constructed in the following manner: The bottom of a plate is covered with a round

slip of filter paper, which should be moistened with water if Müller's mixture is used for fixing, but with 30 per cent. alcohol if alcohol is used. The glass slides are placed on the plate and covered with a large glass bell or with a sheet of glass.

After a few days, the fixing mixture is drawn off by means of filter paper introduced under the cover-glasses; the specimens are rinsed several times with water, and weak (20 to 25 per cent.) alcohol is then added, the specimens being allowed to soak in it for twenty-four hours. The weak alcohol is next replaced, first by 30 per cent., and then by 40 per cent. alcohol, the specimens being allowed, after each change, to remain for some time in the damp chamber. Finally, a drop of glycerine mixed with 40 per cent. alcohol in equal parts is introduced at the edge of the cover-glass. The specimens should be exposed to the air, or covered with a large glass bell, when the water and alcohol will slowly evaporate, leaving the glycerine, more glycerine being added from time to time at the edge of the cover-glass. The objects will finally lie in pure glycerine and will be found sufficiently transparent for examination under the microscope. They should be mounted in glycerine-gelatine in the manner described above. The cover-glass is raised, the glycerine removed, and the glass slide in the immediate neighbourhood of the specimen cleaned with filter paper soaked in weak alcohol. Glycerine-gelatine is liquefied in a spatula over a flame and added to the specimen, which is immediately covered with a clean cover-glass.

Objects fixed with weak alcohol should be treated in a similar manner, omitting, in this case, the water stages. Nematodes prepared in this way will sometimes keep good for twenty years. Looss[1] also recommends the glycerine method for preserving Nematodes. He uses 70 per cent. alcohol, to which, in the case of delicate specimens, 2 to 3 per cent. of its volume in glycerine is added, though a proportion of 5 to 10 per cent. may be employed for the robuster forms. The mixture is heated before use and is poured over the specimens, which should be arranged upon glass slides or in shallow vessels. The worms will die almost immediately and will straighten themselves out, though Trichocephales, Trichosoma and Strongylides, with longitudinal striation of the skin, usually roll themselves up. This can be prevented only by mechanical means.

If Nematodes which have been killed in glycerine-alcohol are placed in shallow dishes, together with a sufficient quantity of the liquid, and covered with a large glass bell, the alcohol will slowly evaporate, leaving the worms in pure glycerine. The process may be hastened by the use of an incubator.

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[1] Looss, *Zool. Anz.*, xxiv, 1901, p. 315.

Langeron[1] uses lactophenol (2 parts glycerine, 1 part distilled water, 1 part crystallized carbolic acid, and 1 part lactic acid). The Nematodes are killed with diluted formol (5 : 100), and then transferred to lactophenol which has been previously diluted with an equal quantity of water, and in this they are allowed to remain for a few hours. A large drop of lactophenol is dropped on to the middle of the glass slide, the worms are placed in it and covered with a cover-glass. The whole of the space under the cover-glass is then filled with lactophenol; the corners are cemented with a drop of glycerine-gelatine (2 parts gelatine, 6 parts water, 7 parts glycerine), the edges are painted with the same material, and, after the gelatine has hardened, they are finished with a couple of coats of good varnish.

(4) *Acanthocephales.*—Although they occur but rarely in man, the study of Acanthocephales should not be neglected. Small species, suitable for whole preparations, are found in the intestine of fish, frogs, water-birds and waders. As a general rule, they are firmly fixed in the substance of the intestinal wall by means of hooks with which the proboscis is covered, and are to be released only by very careful measures. Other varieties do not penetrate beyond the mucosa, and these are more easily freed. Occasionally the parasites are found free with retracted probosces, either in the lumen of the bowel or against the bowel-wall. Fresh specimens are generally flabby and fallen together, and they are more or less wrinkled. When put into normal saline they swell up, the skin becomes taut, and the proboscis is frequently protruded. It is inadvisable, however, to wait until this occurs. The parasities should be cleaned with a brush in normal saline, and as soon as they begin to swell they should be laid upon a glass slide, covered with a cover-glass, and gently pressed until the proboscis is protruded. They are then fixed in strong alcohol which is introduced under the cover-glass, while an even pressure is maintained by placing pieces of lead upon the cover-glass; or a small bottle of mercury may be used; occasionally it may be necessary to use a press. The process of fixing occupies only a short time, and the specimens are then cleared in glycerine and embedded in glycerine-gelatine in the manner already described for the preservation of Nematodes. After fixing, they should be transferred to watch-glasses and stained. If a watery solution of the stain is employed, the worms are very liable to swell up again. In that case they must again be pressed between two glass slides, and treated with alcohol in stages of gradually increasing strength. The specimens may be embedded in gelatine after

[1] Langeron, *C. R. soc. biol.*, lviii, 1905, p. 749; *Arch. de paras.*, xii, 1908, p. 153.

glycerine treatment; or, after clearing with creosote, they may be mounted in Canada balsam.

(*b*) Preparation of Sections.

The best fixing mixture for flatworms is a cold saturated watery solution of mercuric chloride, which as a general rule (and this applies particularly to Trematodes and Cestodes fresh from the host), should be used warm after previous boiling. Small parasites (especially those from warm-blooded animals) which cease to move at room temperature, should be put into flat watch-glasses with a little normal saline and, as soon as they are straightened out, hot solution of mercuric chloride should be poured over them. They become fixed almost immediately, and in the course of a few minutes the fluid may be withdrawn by means of a pipette. The specimens should be washed out with a 0·6 per cent. normal saline, to which it is as well to add a few drops of tincture of iodine. They are next put through the alcohol stages, and this will take one to two days. They are stored in 95 per cent. alcohol.

Large specimens, or those which need straightening, should be arranged with the finger or with a paint-brush before pouring the hot solution of mercuric chloride over them. Large Cestodes, if stretched out upon a plate of suitable length, may be readily killed in this manner. They may also be arranged in zigzags on the glass slide, and if the worm is straightened out with the fingers immediately after immersion in the fixing mixture, the contractions will disappear. This manipulation requires the greatest care and it can be performed only while the tissues are still alive. In the case of thick muscular worms, such as *Tænia crassicollis* of the cat, several minutes are required before death ensues. This is because the thin layer of fixing fluid becomes chilled by contact with the cold glass slide, and its action is, in consequence, retarded.

There is yet another method of managing large worms. The entire length of the worm may be lifted in the fingers by its hinder end and then gradually lowered, head first, on to a warm glass plate containing fixing fluid. The worm will at first contract, but, if the temperature is not too high, it will generally straighten out of its own accord as it dies.

Cestodes which have attained a length of several yards should be cut into lengths and each length fixed separately.

In place of mercuric chloride, the following fixing reagents may be employed: ½ to 1 per cent. chromic acid solution, or acetic acid solution of chromium (chromic acid 2 to 2½ parts, acetic acid 1 part, water 1,000 parts); or, acetic acid solution of chromium and osmium

(chromic acid 2·5 gr., osmic acid 1·0, glacial acetic acid 1·0, water 1,000); or, bichromate of potassium 2 to 5 per cent.; or, Müller's mixture (see p. 101), or chloride of platinum (1 : 300); or, chromic acid and chloride of platinum (1 part each to 800 parts water).

It cannot be said that these reagents are superior in any essential particular to mercuric chloride. But, on the other hand, cases may arise—as for instance, where it is desired to produce certain conditions, or for purposes of research beyond the limits of present knowledge—when other and newer methods must be employed. For the beginner, however, the methods already described will suffice. Bichromate of potassium may be found useful for preparing specimens of organs, from Trematodes and Bothriocephales, which contain yolk-glands and yolk-masses. This reagent colours the yolk a deep brown, and a very good general effect is conveyed by specimens prepared in this way. The specimens should be allowed to remain in the stain for several hours; they should then be carefully dehydrated, cleared, and mounted in Canada balsam.

The preparation of Nematodes for section cutting is a more difficult matter. It is scarcely necessary for the beginner to prepare sections of the smaller varieties, as the more important details of their construction are readily discernible in fresh material and in specimens treated with glycerine. The large varieties should, however, be prepared in sections, the most instructive being *Ascaris lumbricoides*. This parasite takes several weeks to harden. It should be soaked in Müller's mixture, the fluid being frequently changed. The specimen should then be rinsed in water, the process occupying several days and the water being frequently changed. The specimen should finally be put through the alcohol stages and, if these are carefully managed, the worm will not shrink. A quicker method and one which is also unattended by shrinkage of the object, is as follows: First soak the worm in Müller's mixture for a few days; then cut into several pieces with a sharp razor; allow the pieces to again soak in the fixing mixture for a few days; rinse in running water for twelve hours; and, finally, dehydrate by means of carefully graduated alcohol stages.

Sections may be cut by hand from the unenclosed specimen; or the material may be embedded in paraffin, celloidin, or soap. Nematodes treated by Looss's method with glycerine-alcohol, and finished in pure glycerine, may be transferred to strong alcohol, cleared in cedar-wood oil, and then embedded in paraffin preparatory to cutting. The penetrative power of the different reagents will be enhanced if fine incisions are made in the specimens.

Other methods of fixing Nematodes recommended by various authors are: mercuric chloride, both alone and in combination;

picro-sulphuric acid; picro-nitric acid; acetic acid solution of chromium. Specimens are finished in accordance with the fixing mixture, and the results are more rapid if the worms are cut into lengths before fixing.

It is essential that the dehydration of Nematodes should proceed by gently graduated stages. Transference to the clearing fluid and to paraffin must also be done by careful gradation. In each case the fluid is changed by means of intermediate stages, the preponderance of the new reagent being gradually increased.

Acanthocephales are prepared in a similar manner. For fixing, Kaiser recommends a saturated solution of mercuric chloride warmed to about 56° to 60° C.; or a 3 per cent. solution of the same salt to which 1 per cent. glacial acetic acid has been added; or a saturated solution of mercuric cyanide (very poisonous); or the following mixture: acid. picronitr. cryst. 1·0, ac. sulf. conc. 10·0, ac. chrom. cryst. 1·0, aq. dest. 1000·0. All these reagents should be used warm (45° to 50° C.). Small objects should be allowed to soak in mercuric chloride solution for five minutes, larger objects ten to thirty minutes. In glacial acetic acid solution of mercuric chloride, large objects take up to one hour; in mercuric cyanide, from a quarter to one hour, according to size; and in the picric acid mixture, fifteen to twenty minutes. When the first fixing reagent is used, specimens should be washed out with a solution of camphor in 60 to 70 per cent. alcohol, warmed to a temperature of about 60° C., the specimens to be left in soak for two to six hours. When the second fixing reagent is used, specimens are rinsed for four to eight hours in running water. In the case of the third fixing reagent, they are washed out in 70 per cent. alcohol. And, where the picrin mixture is used, they are first rinsed for a short time in warm water, and then put into 60 per cent. alcohol for three to four days.

Specimens are dehydrated by means of alcohol in stages of increasing strength; cleared by means of alcohol and benzol in carefully graduated stages to pure benzol; and embedded by carefully graduated stages of benzol and paraffin to pure paraffin.

Hamann fixes his specimens with mercuric chloride solution, or alcohol to which a few drops of solution of chloride of platinum has been added. He recommends the cutting of the worm into convenient lengths in order to facilitate the action of the reagent.

(c) Preservation for Museum Purposes.

A collection of Helminthes will consist in part only of microscopic preparations, such as whole objects, sections, and series of sections. As a general rule, it is not possible to prepare for the microscope all the individuals of a species which are found at one time. It will

frequently be found expedient, however, to preserve the superfluous material.

The best fluid for preserving specimens for museum purposes is undoubtedly alcohol; or, in certain specified cases (Nematodes), alcohol and glycerine. The fixing reagents are identical with those given above, and to these alcohol may be added. If employed with caution, it is very useful for fixing Trematodes and Cestodes, and may also be used for Nematodes and Acanthocephales; 70 per cent. alcohol warmed to a temperature of about 60° is the best. Examples of new or rare species should never all be fixed with metallic salts or with acids. A certain proportion of the individuals should invariably be killed with alcohol, for it is almost certain that the newer methods of fixing, with metallic salts and acids, are liable to change the tissues. In consequence of this the objects become so brittle that, after they have been kept for a certain time in alcohol, it is impossible to handle them. Absolute, or very strong, alcohol, should on no account be used; the best strength is 60 per cent. or 70 per cent., and it should be slightly warmed. For permanent preservation, specimens should be put into 80 per cent. to 90 per cent. alcohol, in cylindrical glass bottles with wide necks. These should be furnished with glass stoppers, not corks, as the alcohol extracts both pigment and acid from cork, and these will in time affect the object. For the sake of convenience, small varieties of worms may be kept in glass tubes closed with cotton-wool, no air being allowed to remain between the surface of the alcohol and the wad. The tubes are stored, wad downwards, in a bottle filled with alcohol. Under certain conditions, larger varieties, such as Filariæ and thin Cestodes, are also enclosed in tubes before putting into bottles, though this is unnecessary in the case of the robuster sorts. Long Cestodes may be coiled spirally round a tube; the head and tail ends should be fastened with a thread, and the whole then enclosed in a cylindrical bottle containing alcohol.

Dry preparations of large Cestodes are very instructive and are useful for purposes of demonstration. The worms should be cleaned and, if not already dead, they should be killed in weak alcohol. They are laid upon a sheet of black glass to dry and carefully protected from dust. Worms with a large proportion of calcareous bodies in the parenchyma make very striking specimens, as they dry chalk-white. The objects may be kept in square glasses, or they may be framed like pictures.

There is a quick method of preserving intestinal worms in large numbers, which is particularly useful when travelling, or when some chance puts a collector in the way of specimens which interest him. The bowel is opened up along its length; large worms are removed

and, pending ultimate preservation, are put into normal saline solution. The bowel-wall is carefully cleared, the contents being taken out in small portions with the spatula. Epithelium and villi are removed with the fæces only when Helminthes are known to lie, or are suspected of lying, between them. Portions of fæcal matter, of a size varying from that of a hazel-nut to that of a walnut, are put singly into test-tubes. These are then filled for about a third of their length with normal saline and are well shaken for half to one minute, the orifice being closed with the thumb. About the same quantity of saturated solution of mercuric chloride is then immediately added to the liquid, and the tubes are again shaken for about half a minute. The worms usually become extended, and they may be separated from the substance containing them after several days, or even after several weeks. In the latter case, the material should be transferred intact to bottles, which should then be closed, labelled, and stored for future use.

To separate the Helminthes, well shake the material and transfer it to glass tubes. Fill the tubes with water to within a few cubic centimetres of the top. Close the orifice with the thumb and shake gently until the preserved material is evenly mixed with the water. If the tubes are now placed upright in a rack, the Helminthes will sink to the bottom and the dirty fluid may be poured off into shallow bowls. The tubes are again filled up with water, inverted, and placed upright in the rack as before, allowing the worms again to fall to the bottom. This process is repeated until the water remains clear. It very often happens, however, that the bowel contents contain substances which, owing to their specific gravity, sink to the bottom as quickly as, or even quicker than, the worms. As soon as the material in suspension has been got rid of, these heavier particles must be removed. The deposit is turned out into shallow glass bowls and thinned with water. The worms are taken out with the spatula or a paint-brush and dropped into alcoholic solution of iodine. They may be arranged according to size and appearance. The thick fluid is poured off the deposit and this, in its turn, is diluted with water. Shallow vessels should be used and the material must be minutely examined, as there are certain small and fragile varieties which remain for a long time in suspension.

Looss,[1] who was the first to employ this method, says that very small worms sometimes remain between the villi, and that as, after preservation, worms and villi sink to the bottom together, the isolation of the worms becomes a difficult matter. He suggests that the bowel-wall should be cut into pieces, put into glass tubes with some normal saline solution, and the tubes well shaken, when the worms will

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[1] Looss, *Zool. Anz.*, xxiv, 1901, p. 302.

usually emerge from their hiding place. The pieces of bowel-wall should now be removed from the tubes and the liquid treated with sublimate in the manner already described. The tendency of Nematodes to swell under the influence of the normal saline may be counteracted by the use of a stronger (1 to 1·2 per cent.) solution.

Lühe[1] omits the normal saline and applies cold, saturated solution of mercuric chloride directly to the tubes containing the fæcal material. The tubes are then well shaken, closed with a cork, and allowed to remain in a horizontal position. Owing to their specific gravity the worms soon fall to the bottom, and Lühe believes that they stretch themselves better with the glass in this position. Cestodes up to 15 cm. in length may be fixed by this method and will be found well extended. The further treatment is identical with that described above.

CHAPTER III.

EXAMINATION AND PRESERVATION OF THE EGGS OF HELMINTHES.

Generally speaking, the presence of Helminthes in the excretory organs is to be established only by direct examination of the excreta. Mature parasites, or portions of them, are less frequently encountered than their ova. These differ so widely in form, size, colour and contents, that the beginner will very soon find himself able to identify a large number of varieties. This branch of helminthology has such a definite practical value that the student cannot too early familiarize himself with the details of its technique. Good subjects for a first examination are the following: The liver-fluke (*Fasciola hepatica*), which is readily obtainable from abattoirs, as it is frequently present in the livers of sheep; one of the large-hooked varieties of Tæniæ, such as *Tænia crassicollis* of cats, *T. serrata*, *T. marginata*, and *T. cœnurus* of dogs, and, wherever possible, ripe proglittides of *T. saginata* of man; one of the species of Bothriocephalides found in mammals, waders, water-birds, or fish;[2] *Ascaris lumbricoides* from swine or from the horse, which may be obtained from abattoirs; and one of the Acanthocephales from fish, frogs, or swine.

Fig. 30 shows the position of the uterus in the liver-fluke. Its folds form a rosette lying immediately behind the large ventral sucker, which is placed in the median line at the base of the head-cone. A portion of the body mass in this neighbourhood is cut away and teazed out on a glass slide. In the process, a large number of eggs

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[1] Lühe, *Centralb. f. Bakt., Paras. und Inf.*, xxx, pt. 1, 1901, p. 167 (note).

[2] For hosts which are readily obtainable, see Linstow's "Compendium."

will fall out of the uterine folds and these may be examined at once. Young eggs have a colourless shell, old eggs a yellowish-brown one. The contents of both consist of large yolk-cells, which frequently cover the already fertilized, but as yet ungrooved, germ-cell (fig. 31).

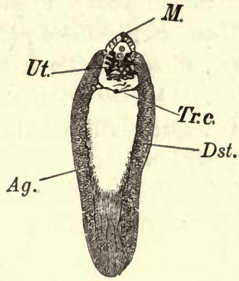

Fig. 30.—*Fasciola hepatica*, L. *Ag.*, Yolk-duct leading from yolk-glands (*Dst.*). *M.*, Oral sucker. *Tr.c.*, Transverse yolk-ducts leading from yolk-glands. *Ut.*, Uterus. (Natural size.)

Fig. 31.—Egg of *Fasciola hepatica*, L. The germ-cell is seen close to the pole at which the lid is placed.

Careful examination under a strong glass will usually reveal the shell-lid, shaped like a watch-glass and placed at the extremity nearest to the germinal cell. Slight pressure on the cover-glass will cause a certain proportion of the eggs to throw back, or at least to lift, their lids.

The eggs of other endoparasitic Trematodes (figs. 32, 33) may differ from those of the liver-fluke in the following particulars: (1) In form; (2) in size; (3) in the possession of a thread-like attachment to the shell at one or both poles; (4) in contents, the egg being deposited at a developmental stage (*e.g.*, with the fully formed miracidium or larva) which will vary with the species; (5) in the absence of the shell-lid; and (6) in the possession of a thorn-like process at one side, or at one end, of the shell. The eggs of Trematodes are very liable to be mistaken for Coccidia or the eggs of Bothriocephales.

Fig. 32. — Egg of *Opisthorchis felineus* (Riv.). 830 : 1.

Fig. 33.—Egg of *Dicrocœlium lanceatum*, St. et H. On the left, lying on its flat surface; on the right, lying on its side.

In the case of the large-hooked worms and the hookless varieties related to them, what are usually described as eggs are finished embryos or oncospheres. These have already lost the true egg-shell,

but are furnished with a covering, composed of rods arranged radially, (fig. 34), which is derived from the substance of the embryo itself. The oncospheres are easily obtained by pricking or teazing out ripe proglottides from the Tænia varieties given above.[1] Eggs covered with a thin colourless shell, to which one or two filaments are attached, are found in the uterus of younger proglottides (fig. 35).

The eggs of other Tænia varieties, when taken from ripe proglottides, also contain fully developed oncospheres. But the shells and embryonal coverings vary in form and number as well as in their arrangement, the different orders and species presenting considerable

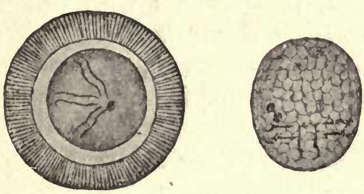

FIG. 34. — *a*, Oncosphere (so-called Tænia egg) of *Tænia african,a* v. Lstw., surrounded by the embryonal covering. *b*, Free oncosphere of *Dipylidium caninum* (L.). (Magnified.)

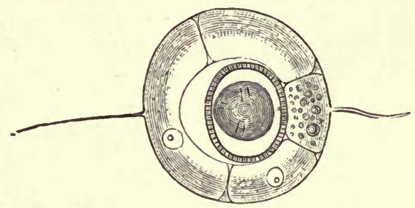

FIG. 35.—Egg from the uterus of *Tænia saginata*, Gze. Thin shell with filaments. In the centre is the ball-shaped oncosphere, surrounded by the embryonal covering with radial markings. The large cells between it and the egg-shell form a second embryonal covering. The granular mass to the right is composed of yolk-granules. 500 : 1. (After Leuckart.)

diversity. An interesting example is provided by the "cucumber" tapeworm (*Dipylidium caninum*), whose eggs, when taken from the ripe proglottides, are arranged in bundles (fig. 36).

There is also considerable variation among the eggs of Bothriocephalides. As a general rule, species with the genital openings upon the flat surface of the body have eggs which resemble those of the endoparasitic Trematodes, the oncospheres being undeveloped even in ripe excreted proglottides (fig. 37). Those species, on the other hand, which have their genital openings at the edge of the body surface, form thin-shelled, lidless eggs, in which the embryo is fully formed before the eggs are deposited.

There is a similar diversity among the eggs of Nematodes. Thus,

[1] Care must be taken to guard against infection when working with ripe proglottides of *T. solium* and *T. saginata* of man, as man may also serve as the intermediate host for the development of the oncospheres into Cysticerci. Similarly, precautions should be observed when making the *post-mortem* examination of dogs, as the oncospheres of *T. echinococcus* will also develop in man. This worm is very small and very difficult to find, as it conceals itself between the villi.

the eggs of *Ankylostoma duodenale* are oval and thin-shelled, and are deposited, either before the germinal groove appears, or while it is still in a very early stage of development. The eggs of *Oxyuris vermicularis,* on the other hand, though oval, are furnished with a thick shell and contain an embryo resembling a tadpole (fig. 39). This embryo, when subjected to sufficient heat, passes in the course of a few hours into a nematoid stage of development. The degree of difference which may subsist between closely allied species, is shown by a comparison of the eggs of *A. lumbricoides* with those of *A. canis* (= *A. mystax*) (fig 40).

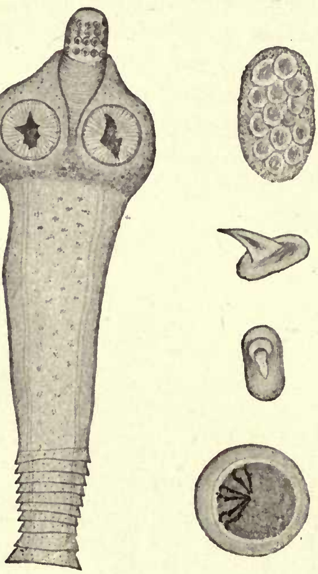

FIG. 36.—*Dipylidium caninum* (L.). Left, scolex; right, at the top, a bundle of eggs; underneath it, hooks from the rostellum, seen from the front and in profile; at the bottom, an egg. (After Diamare.)

Unfertilized eggs of Ascarides are frequently found in the stools of man. These differ from normal eggs; firstly, in their shape, which is longer; secondly, in their contents, which are richer in yolk-granules; and thirdly, in the manner in which the germinal cell entirely fills the shell. *Eustrognylus gigas* (fig. 41) is distinguished by the peculiar thickness of its shell, which, except at the poles, is of a brownish colour and is very much pitted. The eggs of *Trichocephalus* are also oval, but they are flattened at the poles

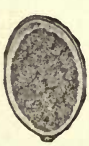

FIG. 37. — Egg of *Dibothriocephalus latus* (L.). 240 : 1.

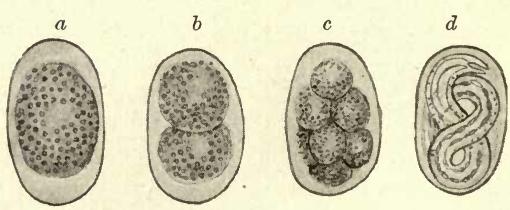

FIG. 38.—Eggs of *Ankylostoma duodenale* in different stages of development. *a*—*c*, In fresh fæces. 336 : 1.

and appear barrel-shaped. The shell is thick, brownish in colour, and perforated at the poles, the openings being closed with a light mass. These eggs are deposited before the developmental changes commence, the shell, in this case also, being entirely filled by the granulated germ-cell (fig. 42).

The eggs of Acanthocephales are very characteristic. They are spindle-shaped, though sometimes oval, and are enclosed in several transparent shells of different thicknesses. When deposited they contain a finished embryo, of which, however, the hook is the only recognizable feature (fig. 43).

FIG. 39.—Egg of *Oxyuris vermicularis*, L. 640 : 1.

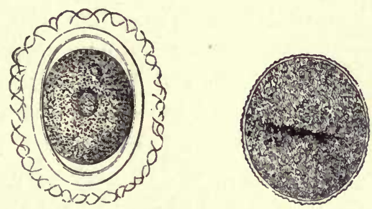

FIG. 40.—Left, egg of *Ascaris lumbricoides*, L. 400 : 1. Right, egg of *Ascaris canis* (L.).

Eggs of Helminthes should be preserved on a glass slide, the cover-glass being raised by means of supports of a suitable thickness. They are fixed in alcohol, which should be repeatedly changed and is finally

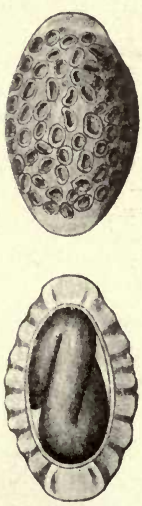

FIG. 41.—Egg of *Eustrongylus gigas* (Rud.). The upper figure shows the flat surface; the lower figure shows the egg in section.

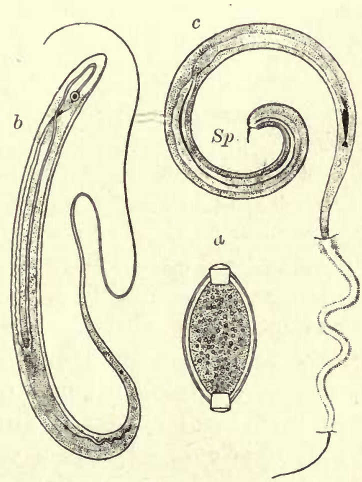

FIG. 42.—*Trichocephalus trichiuris* (L.). *a*, Egg, very much magnified. *b*, Female. *c*, Male. *Sp.*, Spiculum. (Slightly magnified.) After Claus.)

replaced by a mixture of glycerine and alcohol. If carefully treated the eggs rarely wrinkle. The glycerine and alcohol stages are carefully graduated until the eggs lie in pure glycerine, which is replaced with

liquefied glycerine gelatine. When cold, the edges of the cover-glass are painted with varnish. The entire process may be carried out in a watch-glass, if preferred.

The method of examining the excreta of persons suspected of harbouring Helminthes, varies with the consistency of the secretion. Where the fæces are watery, the liquid should be allowed to stand for a short time in glass bowls or tubes, and portions of the sediment should be taken out with a pipette and examined. It is sometimes necessary to pour off the liquid and centrifugalize the sediment. Solid fæcal matter should be reduced to a suitable fluidity by the addition of some indifferent liquid (such as normal saline) allowed to stand for a time, and then treated in the manner just described. Or small portions of solid matter may be liquefied in watch-glasses with normal saline, rubbed down, allowed to stand, and the sediment then examined. It is hardly necessary to say that a single test is insufficient to establish a diagnosis. A very large number of tests must be made before it is possible to declare definitely that Helminthes are not present.

FIG. 43.—EGG of *Echinorhynchus gigas*, Gze. 300 : 1. (After Leuckart.)

Looss[1] recommends the following method of preparation: If the fæcal matter is too thick it should be rubbed down in watch-glasses to the consistency of thin paste, and any coarse particles should be removed. A mixture consisting of 100 parts 70 per cent. alcohol, with 5 parts glycerine, is heated in a bowl in a water-bath to very nearly boiling point. The material for examination is then added to the hot liquid, carefully stirring all the time. When cold, the liquid, which will now have assumed a yellow colour, is poured off, without, however, disturbing the sediment, and is replaced by fresh liquid. The alcohol is allowed slowly to evaporate, a thermostat at a temperature of 50° being used for the purpose. At the end of one to two days the fæcal matter will be evenly distributed through the pure glycerine, and may be kept for future use. For microscopic purposes, it will only be necessary to place a small drop of the sediment upon the middle of a glass slide containing a small quantity of warm glycerine gelatine. The drop of fæcal matter is stirred into the glycerine gelatine, the preparation is covered with a cover-glass, and, when cold, the edges are painted round with varnish.

[1] Looss, *Handb. der Tropenkrankh.*, i, 1905, p. 39.

CHAPTER IV.

REARING HELMINTHES—FEEDING EXPERIMENTS.

THE progress which has recently been made in Helminthology is, to a large extent, the outcome of experiments in hatching and rearing Helminthes. These experiments are undertaken with the object of following the life-history of the worm through its various developmental stages. As a general rule, Helminthes do not live in the open, and the only way to develop them is by reproducing the conditions under which they normally thrive. In other words, they must be reared within the body of a suitable host. At a certain stage of their development, the worms, either with or without a vehicular mass, are introduced by the mouth into the body of an animal of a certain species. The different varieties of Helminthes are parasitic in certain definite host-species only and, if introduced into the bodies of other hosts, they fail, as a rule, to develop. To discover the precise host-species in which a certain stage of Helminth will continue its development is by no means an easy or a simple matter. The beginner will do well to confine himself to experiments, the result of which has been already ascertained.

The first experiment of the kind was carried out at the end of the eighteenth century by P. C. Abildgaard. The striking similarity between the immature tapeworms so frequently encountered in the body-cavity of the stickleback (*Gasterosteus aculeatus*) and the mature forms found in the intestine of water-birds, led him to suspect that the tapeworm of the stickleback attains maturity and deposits eggs, only after it has reached the intestine of the water-bird. At that time, and indeed much later, sexually immature Helminthes were universally regarded as of separate species, and experiment alone could determine whether the suppositions of Abildgaard were justified by facts. To put the matter to the test, two farmyard ducks were for some days fed with sticklebacks. Upon examination, the intestines of these ducks were found to contain worms in the sexually mature form, exactly similar to those found in the bodies of wild ducks, which are known to feed upon sticklebacks. The condition of sexual maturity was undoubtedly brought about by a few days' sojourn within the body of a fresh host of suitable species.

Although this experiment established a precedent, showing as it did the connection between the mature tapeworm and a young form resembling it, other experiments of the kind were not made. It was not until 1851 that F. Küchenmeister again made use of the method, and since that date it has proved of the utmost value in practical Helminthology. By means of experiments similar to those of

Abildgaard, intermediate stages may be traced and the entire life-history of the worm, from the egg to the mature adult, may be followed. Generally speaking, three stages are recognized in the development of Helminthes: the embryo; the intermediate, young, or immature worm; and the sexually mature, adult parasite. Of these three stages, the two latter are developed within the bodies of separate hosts, which are known respectively as the "intermediate" and the "definitive" hosts. As the embryo does not usually develop until after the egg is deposited and, frequently, not until after it has left the body of the definitive host, the egg must be hatched out by artificial means. This is usually an easy matter. All that is required is to place the eggs, as soon as possible after they are laid, under conditions favourable to their development. Or eggs may be used which have been removed from the uterus immediately before deposit.

Eggs which, according to species, have been deposited either before or in the course of cell-division, will develop in water or damp earth; those of Cestodes and Trematodes in water, those of Nematodes in damp earth or fæces. Thus, the eggs of the broad tapeworm (*Dibothriocephalus latus*) or of the liver-fluke (*Fasciola hepatica*) should be removed from the fæces or from the terminal portion of the uterus, put into shallow vessels containing filtered water, covered with a glass plate, and allowed to remain in a well-aired room. The period necessary for the development of the eggs varies with the temperature and the lighting. In summer, development occupies a few weeks only; in spring and autumn, it takes longer; while in winter, unless an incubator is used, it is arrested altogether. Cultures of this kind sometimes fail owing to the development, in large quantities, of bacteria or other vegetable or animal parasites. Or it may happen that the eggs are unripe. In the case of the liver-fluke, the latter contingency may be guarded against by using for experiment those eggs only, which have been taken from the gall-bladder of the host.

Failure is still more frequent in the case of Nematode eggs hatched in damp earth. The material should be placed in watch-glasses, or in shallow glass or porcelain bowls, and carefully covered, without, however, being rendered air-tight. They are then put under an inverted funnel, the stem of which is closed with a wad of cotton-wool. The best medium is common garden mould, which has previously been heated in order to kill all germs and animalculæ. When cold, it should be moistened with filtered water (though boiled water is better) and it should be kept wet. The eggs of Nematodes may also be developed between wet blotting paper. In the case of many varieties, as, for instance, Trichocephales, the eggs have such an immense power of resistance that they will develop in the reagent

which is used to preserve them, and are frequently undamaged by drying.[1]

For cultures in fæces, especially of the eggs of *Ankylostoma duodenale*, Looss[2] recommends the admixture of an equal quantity of bone-black. This not only removes all odour, but it also favours the development of the worms. Water should not be added, but the culture should be frequently well stirred in order to bring the eggs which are at the bottom into contact with the air.

The manner in which the embryo leaves the shell, or other covering, varies considerably. Some varieties, whether formed before or after the deposit of the egg, cannot be induced to emerge in the open. Such are those of the Acanthocephales, the majority of the Cestodes, and many Trematodes and Nematodes (Ascaris). The embryos of certain other varieties, such as those of the liver-fluke (fig. 44), and the broad tapeworm, emerge readily and move about in the water or earth (Nematodes) of the culture. In the case of embryos which emerge in the open, their free existence may last for a comparatively long time. The young worms feed, grow, cast their skins and, in the case of one group (Angiostomides), become sexually mature. This instance of heterogony is extremely interesting, and the process may be watched by means of experiments in the following manner.

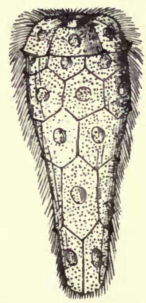

FIG. 44.—Miracidium of the liver-fluke immediately after leaving the egg. Magnified. (After Leuckart.)

A small roundworm, *Rhabdonema nigrovenosum*, belonging to the Angiostomides, is found in the lung of the brown frog. As soon as the eggs are deposited, they make their way through the branches of the lung to the oral cavity. They are then swallowed by the frog and thus find an entrance into the alimentary canal. As they traverse it, the development of the embryo becomes complete, and the young worms usually emerge from the shell—which is oval in shape and very thin—in the terminal portion of the large bowel. The number of eggs deposited is so large that, if *Rhabdonemæ* are present in the lungs, eggs containing embryos, or young worms which have left the cell, are certainly present in the lower bowel. These young worms are about 0·4 mm. long and are furnished with a double œsophageal swelling. They may be brought to sexual maturity in the following manner.

[1] There is no fixed line of demarcation between water and earth cultures. The eggs of Trichocephales, for instance, develop in water as well as in mould; those of the liver-fluke will develop in mould if it is wet enough; and those of Ascarides do equally well in either medium.

[2] A. C. Looss, *Centralb. f. Bakt., Paras. und Inf.*, xx, 1886, p. 866.

The contents of the lower bowel of an infected frog are carefully freed, with the aid of a magnifying glass, from other parasites (larger Nematodes and Trematodes). The mass is then transferred in small portions to a watch-glass, the surface of which has been previously covered with damp earth. The glass is covered over and put into a damp chamber. If the external temperature is high, the young worms will slough their skin and attain sexual maturity in about a day. In the winter, on the contrary, the process may occupy a week. The body is extremely transparent and allows the internal organs to be easily examined. The smaller male, which is recognized by its spicule (fig. 45), dies soon after copulation, while the fertilized eggs develop in the uterus of the female. The young worms emerge from the shells, break through the uterine wall, and grow up within the body-cavity of the mother, whose internal organs they completely destroy. At the end of four to five days, in summer, nothing is left of the mother but the cuticle, in the interior of which the worms make lively movements. As soon as they leave the cuticle and reach the open air, their rhabditis form, which is characterized by two pharyngeal swellings, becomes modified. After that they do not change. They wait until an opportunity offers, when they make their way into the larynx of a frog and thence into the lungs. This may be accomplished artificially, by transferring a portion of the slime containing the modified young worms to the mouth of a frog. The swallowing of the mass is prevented by holding the mouth open, while the entrance to the lung which is situated upon the ventral side at the base of the larynx, is held apart with tweezers, in the expectation that some of the worms will find their way into the lungs. Though, of course, not invariably successful, this experiment is attended by results in the majority of cases. If the slimy mass is swallowed the worms will die in the stomach of the frog.[1]

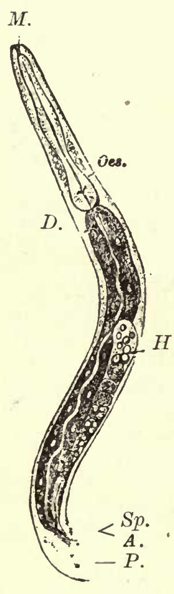

FIG. 45.—Male of the free-living generation of *Rhabdonema nigrovenosum*. Magnified. (After Leuckart.) *A.*, Anus. *D.*, Small intestine. *M.*, Mouth. *Oes.*, Œsophagus. *P.*, Papillæ at the tail-end. *Sp.*, Spicula.

A similar developmental process, consisting in an alternation of parasitic and free-living generations, is observed in the tropical form

[1] R. Leuckart, "Die menschl. Parasiten," vol. ii, Leipzig, 1876, pp. 139-148.

of *Strongyloides stercoralis*[1] (Bavay), which inhabits the intestine of man. In the form found in Southern Europe, which is occasionally imported into this country, the free-living generation is usually absent. This is to say, the young worms of the parasitic generation do not attain sexual maturity and do not multiply after leaving their host. When in the open they transform themselves into larvæ, whose further development is dependent upon their importation into man.[2]

Looss[3] describes a method of isolating Nematode larvæ, which is very useful in the case of worms reared in fæces. He carried out the method with *Ankylostoma*, but it may be employed for other parasitic larvæ. The fæces are first mixed with bone-black and then allowed to stand, the mixture being occasionally stirred. The period of development depends upon external temperature. With a high reading of the thermometer, such as summer temperature in Egypt, the surface of the culture will be covered with newly hatched larvæ in twenty-four hours. The culture is then exposed to the air until the surface is covered with a thin, but firm, crust. If the vessels are now filled up with clean water and allowed to stand for ten to twenty minutes, the larvæ will appear in large numbers in the water, and may be poured off with it. The culture should be re-dried, and the process repeated, until the larvæ cease to appear in the water. To insure absolute isolation, the water which has been poured off the culture, and which will contain fine particles of fæcal matter, should be filtered. The water which first runs through the filter will be discoloured, and should be thrown away as soon as the water begins to run clear. The clear liquid will be found to contain larvæ which have bored their way through the filter paper, and these will appear in increasing numbers. They will be found most abundantly, however, if the empty filter is allowed to stand in the funnel for about twelve hours, and clean water is again passed through it. This process should be repeated until larvæ cease to appear in the filtered fluid. This method has also been found useful in isolating the larvæ of *S. stercoralis*[4] and should be applicable to many other species.

The water containing larvæ may be used to infect animals of suitable species, and the details of the experiment will vary according to the nature and habits of the Helminthes used. Those which develop directly in a definite host (many Nematodes) will require a

[1] The Strongyloides of man should be examined with the greatest caution, as infection with free larvæ is possible, not only by way of the mouth, but by way of the skin. This applies also to *Ankylostoma duodenale*.

[2] See Braun-Seifert's " Parasitenwerk," 4th ed., Würz., 1908, p. 288.

[3] Looss, *Centralb. f. Bakt., Paras. und Inf.*, part 1, vol. xxi, 1897, p. 914.

[4] Braun, *ibid.*, part 1, vol. xxvi, 1890, p. 614.

different treatment to those which demand the agency of one or more intermediate hosts before attaining sexual maturity. In the management of all such experiments, however, there are certain precautionary measures which must be observed. It is of paramount importance to make sure that the animal to be used as a host is free from Helminthes; or, at any rate, from those which form the subject of the experiment. Repeated microscopic tests of excreted material will show whether eggs are present or not; but the absence of eggs from the excreta is by no means a certain proof of the absence of Helminthes from the excretory organs. It may happen that the animal harbours parasites which have not attained sexual maturity and which do not, as yet, deposit eggs. Where infection is proved, it is rarely possible to free the animal from worms, and, in any case, anthelminthic measures could only take effect upon worms present in the intestine. Again, the employment of young animals and sucklings does not guarantee more than freedom from parasites, which might have been present in media to which the animal has been denied access. It does not safeguard against infection by swallowing insects or other small animals, or against infection with developmental stages (eggs containing embryos, larvæ) present in the excreta of the mother. Suckling kittens and puppies, for instance, frequently harbour *Dipylidium caninum*, transmitted by ectoparasitic insects, and *Ascaris canis*, the eggs of which they have swallowed.

The only possible method is to keep the animal isolated under conditions of the most scrupulous cleanliness, and to feed it upon perfectly untainted food. This, however, is more easily said than done. Better results will be obtained if several animals are used for experiment at the same time and examined at regular intervals; or a single individual may be treated with infective material over either a prolonged period or several short ones, the examination being made soon after the last infection. It is possible by these means to obtain the parasite in its successive developmental stages, from the early stage used as the agent of infection to the final adult worm. If, during the entire course of the experiment, the possibility of infection by other means is carefully guarded against, these results should be conclusive. It is not always easy to prevent spontaneous infection, but every precaution should be taken. The animals should be housed singly in cages which are easily cleaned out, and which must be scrupulously kept. Their food and drink must be above suspicion. The domestic animals are most easily managed, especially those which have been reared in captivity through several generations. Unfortunately, however, their uses are limited, as they can be used for experiment only when they happen to be the right host for the particular species of parasite under investigation.

Where it is desired to obtain intermediate encysted living stages from certain hosts, the microscopic examination of the excreta is naturally useless. Infection is never proved by this means and other measures are successful in a small proportion of cases only. Thus, trichinosis may be diagnosed by examining under the microscope small portions of the muscular structure taken from the living animal. The results are here, to a large extent, dependent upon chance, although the probabilities of success will be increased by frequent repetition of the tests, or by the finding of stages in process of transformation.

It must not be forgotten that, when handling Helminthes which are parasitic in man, there is considerable danger of self-infection unless suitable precautions are employed. Moreover, the beginner is earnestly warned against all experiments with man as a host. These should be left to experienced scientists who are in a position to guard against the risks inseparable from such undertakings.

In the great majority of cases, infection with Helminthes takes place by the mouth, though in a few isolated instances it may occur by way of the skin (*Ankylostomum strongyloides*). In cases where the life-history of the parasite is already known, the host-species into which it should be introduced is readily ascertainable. This applies also to those parasites (many Nematodes) whose history does not include an intermediate infective stage. But where the intermediate stages, or the hosts in which such stages develop, are not known—or where these are known, while the definitive host is unknown—the finding of an animal of suitable species in which to carry on the experiment is attended by difficulties which are frequently insurmountable. In cases such as these, we are forced to fall back on the results of previous experiment and, after duly considering all the species which, by their life-history and nutritional habits, might serve as intermediate or definitive hosts to the parasite under consideration, to select the one for experiment which appears to offer some likelihood of success. The conclusive test is furnished by the results of the experiment. Occasionally, however, the introduction of parasites into a host of suitable species is unattended by success. Failure in such cases is due to some technical error, or to changes, the significance of which has not been understood, in the nature of the infective material itself.[1]

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[1] Thus R. Leuckart was unsuccessful in infecting himself with *Ascaris lumbricoides* by means of eggs containing embryos. This led to the erroneous supposition that *A. lumbricoides* required the agency of an intermediate host before attaining its final development in man. The failure was due, however, to quite another cause. The eggs which were used for experiment had lost their albuminous envelope, with the result that, on reaching the stomach, the embryos which they contained died. The observations of other authors show that, had the albuminous envelope been perfect, infection would most certainly have resulted.

The Trichocephales (*T. affinis* of sheep, *T. crenatus* of swine, *T. depressiusculus* of dogs, *T. unguiculatus* of rabbits and hares, *T. nodosus* of rats) supply material for direct infection by means of eggs containing embryos. The eggs should be removed from the uterus and cultivated in water or damp earth. As a rule, development proceeds slowly and many eggs perish in the process. As soon as the embryos are formed, the eggs are mixed with a suitable vehicle, such as milk, sop, &c., and introduced by the mouth into the animal selected for the host. According to Leuckart and Grassi, Trichocephales will be fully formed at the end of four to five weeks and may be seen microscopically in the cæcum. The young forms, which are exceedingly small and only to be detected with the aid of the microscope, will be found in the intestinal mucus. They appear six to ten days after infection—never later, though sometimes earlier—and they will be found in an already infected animal, if a second infection is induced a few days before examination.

The *Ankylostoma* varieties may be taken as examples of parasites, the larval stage of which emerges from the egg and attains a certain stage of development before introduction into the final host. A good substitute for the *A. duodenale* of man is *A. trigonocephalum*, found in dogs and foxes. The dog should be used for experiment. The eggs are best cultivated in the fæces of their host by the method described above, bone-black being mixed with the fæcal material and water used to dilute it. The larvæ are isolated by pouring off the water and filtering it. They invariably fall to the bottom of the water and will remain alive in it for months. They are useful for purposes of infection only after they have shed one skin and are preparing to shed another. This takes place soon after they have left the shell. The ripe larva is recognized by the presence of an envelope, detached at the head and tail, which represents the cuticle immediately before sloughing. Certain changes will also have taken place in the shape of the body and the pharynx. This description applies equally to larvæ of *A. duodenale* of man. According to Leuckart, the sloughing of the larvæ of *A. trigonocephalum* of dogs takes place during the free stage. This parasite, like the species found in man, does not undergo any further transformation until after its introduction into the body of the definite host. This is effected directly, by means of water or earth containing larvæ. If infection is very severe, the host will perish in about ten days. Vomiting sometimes occurs soon after infection, and this is very liable to endanger the result of the experiment. The best hosts are, undoubtedly, young dogs.

The difference in structure, especially in that of the excretory organs, between the larva and the adult parasite is very great and is

effected by means of numerous transformations. These take place during the fourteen days following infection and are characterized by repeated sloughings. Some of the transformation forms are given in fig. 46.

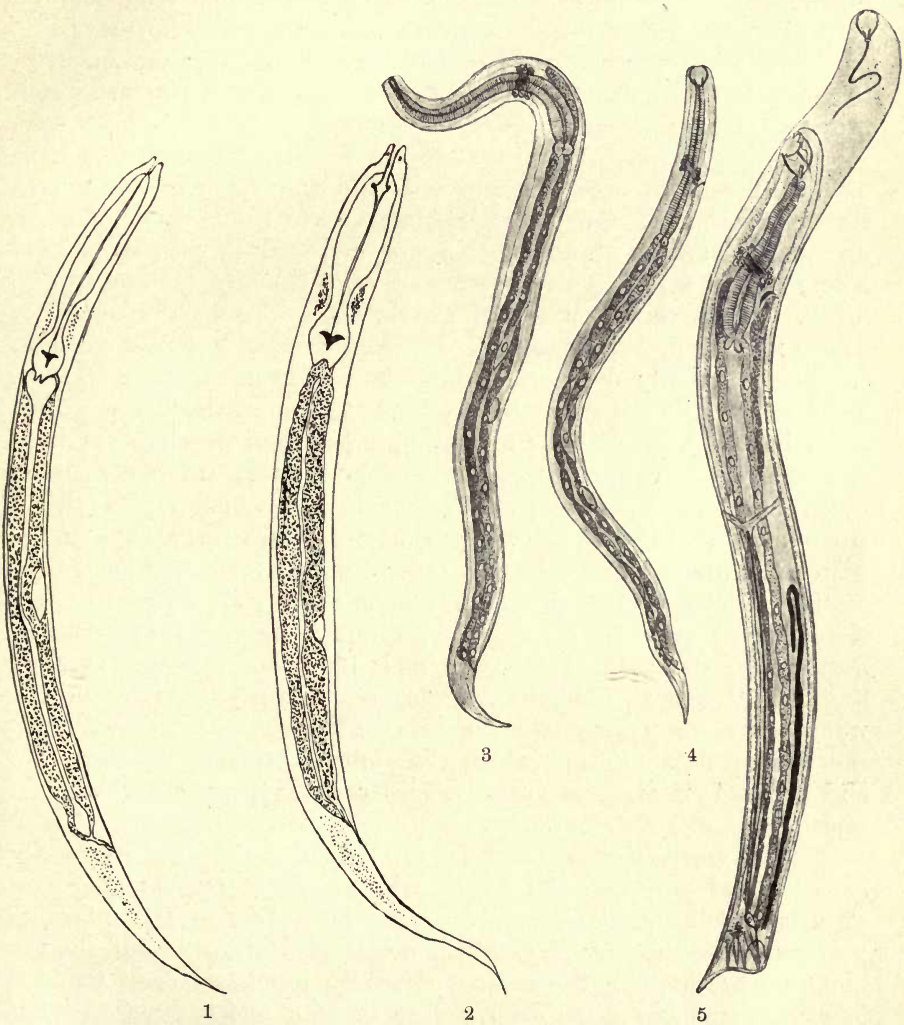

FIG. 46.—1, Larva of *Strongyloides stercoralis*. 2, Larva of *Ankylostoma duodenale*. (After Leichtenstern.) 3, *Ankylostoma duodenale*, four days after its introduction into the body of a dog; 190 : 1. 4, The same, at the commencement of the second developmental stage (5—6 days); 105 : 1. 5, Two weeks after introduction into the body of a dog; the parasite is shown in the act of sloughing; 42 : 1. (After Looss.)

Ankylostoma may also enter their host by way of the skin. Looss proved this conclusively by experiments upon himself with water

containing larvæ of *A. duodenale.* Upon another occasion, a drop of water containing larvæ was spread upon the skin of a living limb shortly before amputation, and the portion of skin which had been wetted was examined immediately after the operation. This experiment, though offering conclusive proof of penetration only, showed very distinctly the manner in which this was effected, the larvæ entering mainly through the hair-follicles and shedding their skin in the process. The experiments which Looss performed upon himself proved, not only penetration but infection also; that is, worms were subsequently found to be present in the bowel. These results have been confirmed by other authors and may be verified by means of a simple experiment. All that is necessary is to bring water or earth containing larvæ of *A. trigonocephalum* into contact with the skin of a dog, and to keep the place covered with a bandage for one to two hours. The larvæ will penetrate the skin of young dogs more readily and in larger numbers than that of full-grown dogs. For this reason puppies frequently die, while older dogs bear infection very well. Upon examination of the intestine, however, comparatively few worms are to be found, the majority appearing to perish in the course of their wanderings. It will, of course, be understood that when introduced by the mouth, the parasites attain sexual maturity more quickly than when introduced by the skin. The larvæ do not ripen except in the bowel and, in the latter case, they only reach it after a long journey.

The Cestodes, the digenetic Trematodes, and certain of the Nematodes require the agency of two, and sometimes three, distinct host-species in order to complete their life-history. There is, first, the definitive host, almost invariably a mammalian, in which the worm attains full size and becomes sexually mature; and second, the intermediate host, in which the embryo or larva develops into an encysted intermediate stage, either directly or after asexual multiplication.

Tænia crassicollis of cats is a Cestode eminently suited for experimental purposes. The Cysticercus stage is passed in the liver of rats and mice. The parasites may be reared in the following manner. The mature eggs, which are formed in vast numbers, are obtained by pricking the uterus of a ripe proglottis from the cat. They are transferred to bread soaked in milk and the mass is used to feed white mice. If the eggs are mature and the mice healthy, the results are almost certain. The dose should be carefully regulated, however, as if too many of the eggs are swallowed the liver becomes very much changed and the mice will die in the course of the second week.

As early as five hours after infection, free oncospheres will be found in, generally, the middle third of the small intestine. At the

end of nine hours, as well as at a later period, they will be found free in the blood-stream in the portal vein. It is a more difficult matter to catch them in the act of perforating the bowel-wall. The best method is to kill the host at the end of the first, or beginning of the second, day; rapidly to sever the small intestine at the mesentery; and to prepare it in series of sections. It will be necessary, however, to examine a large number of sections before finding one which shows the oncospheres within the substance of the bowel-wall. The developmental changes which the oncospheres undergo in the liver, as well as the pathological changes to which they give rise, are best seen by means of sections cut from the affected organ. Where, however, the Cysticerci are solarge that they may be prepared singly, it is better to examine the living object. The worm is of slow growth. Thus, the head does not make its appearance until twenty-five days after infection, while the suckers and hooks do not appear until forty days after infection. A large number of individuals fail to develop and gradually perish, one worm only, though occasionally more, attaining complete maturity.

The *Cysticercus fasciolaris* of *Tænia crassicollis* as very distinctive. After the development of the scolex, they proceed to form a chain of proglittides, which lengthens with age and which differs only from that of the mature worm in having at the tail-end the characteristic Cysticercean bladder. Except that they are without genital organs of any description, the single segments exactly resemble those of the adult worm.

Cysticerci in this stage of development may be introduced into suckling kittens or kittens which have just been weaned. This is done by holding the mouth open and placing a Cysticercus at the back of the tongue, when a swallowing movement on the part of the kitten will propel the parasite into the alimentary canal. One cat may be given several Cysticerci, either immediately following one another, or at longer intervals. In opposition to the prevailing view, E. Bartels maintains that the only part of the Cysticercus which perishes, is the bladder. He believes that not the scolex only, but the entire segmented body, with the exception perhaps of the end-joints, continues its existence in the new host, that it becomes sexually mature, and adds to its length by the formation of new proglottides on to the old ones.

Another subject for experiment, which is easily obtained and convenient to handle, is *Tænia serrata* of dogs, the intermediate stages of which are found as *Cysticercus pisiformis* in the liver of rabbits and hares.[1] *T. marginata* is also obtained from dogs, and here the inter-

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[1] See R. Leuckart, R. Moniez.

mediate host is usually the sheep, in the omentum of which, after leaving the liver, the large bladder-worm, known as *C. tenuicollis*, develops. *T. cœnurus* is another parasite of dogs, the intermediate stages of which are passed in the sheep, the large Cysticerci, which form many scolices, being in this case found in the brain, where they originate the disease known as "sturdy." The dog also harbours *T. echinococcus*, the Cysticerci of which are known as Echinococci. These are characterized by the formation of brood-capsules containing numerous scolices, which develop in the liver of domestic mammals, but of which man is also a host. Experiments with this parasite should be carried out with utmost caution.

The Cysticerci which occur in crustaceans, insects and worms, may also be reared artificially, but the task is a difficult one. The better plan is to examine hosts which have become infected by natural means and, if a sufficient number of individuals are used for experiment,

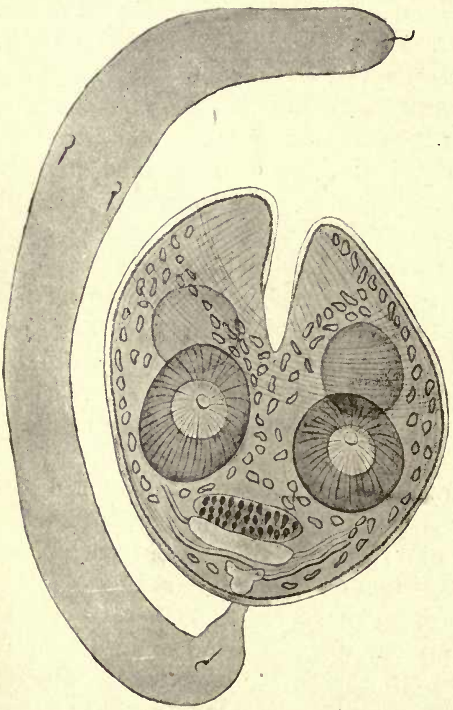

FIG. 47.—Cysticercoid from *Dipylidium caninum*. Very much magnified. (After Grassi and Rovelli.)

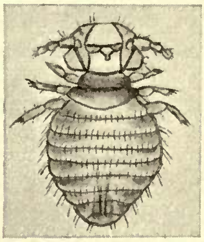

FIG. 48.—*Trichodectes canis* (Deg.). Male. Magnified. (After Piaget.)

Cysticerci in all stages of development will be found. The most convenient subject for experiment is *Dipylidium caninum*, the "cucumber" tapeworm of dogs and cats, the Cysticercoid stages of which (fig. 47) occur in the dog-flea (*Pulex serraticeps*), and in the dog-louse (*Trichodectes canis*, fig. 48). These insects are ectoparasitic upon the bodies of dogs and cats, and, when teazed out, will frequently be found to contain Cysticerci.

Hymenolepis murina, which inhabits the intestine of rats and mice, also passes through a cysticercoid stage, though without the agency

of a fresh host, development taking place within the intestinal wall of the definitive host. This stage may be seen after feeding rats or mice with ripe proglottides of the parasite (fig. 49).

An excellent subject for the study of the digenetic Trematodes is afforded by the liver-fluke, *Fasciola hepatica*, which is readily obtainable from slaughter-houses. Eggs, when taken direct from the uterus, are in varying stages of maturity, and only a certain proportion will develop in water. It is better, for this reason, to use eggs which have been deposited, and these are usually found in large numbers in the gall-bladder of infected sheep. After several weeks the miracidia, which are long in shape and entirely covered with hairs (fig. 44), will emerge from the shell, and should now be brought into contact with the intermediate host. This function is fulfilled by a small fresh-water snail (*Limnæus minutus*, fig. 50), which must be quite young and into which the miracidia penetrate, shedding their ciliated coat in the process. Once within the body of the snail, the intestine and nervous system undergo retrograde changes, and the larvæ become converted into sporocysts. In the sporocysts, rediæ provided with an intestinal apparatus are formed, and from these the cercariæ are developed. The snails should be fed with water plants, lettuce leaves, &c., during the developmental period. When formed, the cercariæ leave their host and swim actively about in the water, where they appear as small whitish bodies. Finally, they cast their tails and, after creeping up the stems of water plants, they encyst above the level of the water.

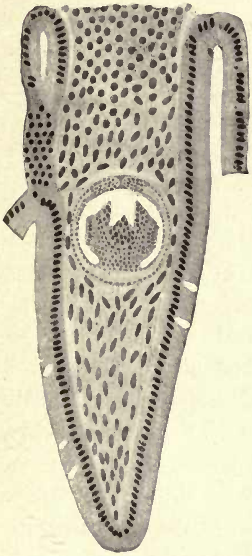

FIG. 49.—Longitudinal section through a villus of the small intestine of a rat with a cysticercus of *Hymenolepis murina*. Magnified. (After Grassi and Rovelli.)

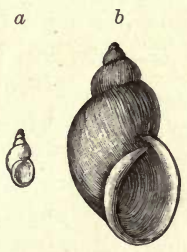

FIG. 50.—Shell of *Limnæus minutus* Drap. *a*, Natural size. *b*, Magnified. (After Leuckart.)

Another subject which is easily procurable is *Diplodiscus subclavatus*, an Amphistomide which is frequently present in the terminal portion of the gut of frogs. The eggs when deposited contain the fully formed miracidia, which soon emerge and penetrate into small varieties of Planorbis. The cercariæ encyst spontaneously in the water or on frogs.[1]

[1] A. Looss, "Festschrift z. 70. Geburtst. R. Leuckarst," Leipzig, 1892.

The eggs of *Paramphistomum cervi* (= *Amphistomum conicum*), which are of such frequent occurrence in the paunch of the ox, are also easily hatched. The miracidia, which are exceedingly active, develop in fresh-water snails of the family Physa.[1]

Much interesting material for experiment is suggested by the current works on Helminthology. The student is offered a wide field for investigation for, although a large number of Helminthes are known to us, the complete developmental cycle has been traced out in the case of but very few.

CHAPTER V.

EXAMINATION OF HELMINTHES AND OF THEIR DEVELOPMENTAL STAGES.[2]

(1) TREMATODES.

THE majority of the Trematodes observed in man are extra-European in their occurrence, and but rarely available for purposes of experiment. Generally speaking, the European varieties are parasitic in man occasionally only, their normal hosts being the domestic mammals, and this applies to many extra-European varieties. Thus, *Fasciola hepatica* and *Dicrocœlium lanceatum* are normally parasitic in the sheep; *Opisthorchis felineus* in the cat. All three species inhabit the gall-ducts of their host, but they vary in the frequency of their occurrence. The commonest is undoubtedly the liver-fluke (*F. hepatica*), which is practically always obtainable in Central Europe from the larger abattoirs. The lancet-fluke (*D. lanceatum*) is also to be obtained from slaughter-houses, though it is of somewhat less frequent occurrence than *F. hepatica*. *O. felineus*, the fluke of the cat, is confined in Germany almost exclusively to West and East Prussia. It occurs in other hosts besides the cat in France, Holland, Scandinavia, Russia, Hungary, Italy, Siberia and Japan.

The three European varieties are not equally valuable as subjects for experiment. The liver-fluke, though readily obtainable, is of but slight transparency and has considerable complexity of structure, these characteristics rendering it a somewhat difficult subject for the beginner. The lancet-fluke and the fluke of the cat are, when obtainable, better subjects for a first experiment. On account of their trans-

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[1] A. Looss, "Mém. de l'Inst. égypt.," vol. iii, 1896.

[2] Before making practical use of the information contained in the following chapters, the student is advised to study the subject in M. Braun's "Animal Parasites of Man." For Cestodes and Trematodes, Bronn's "Klass. u. Ord. d. Tierreichs" should be consulted.

parency they are readily examined in the fresh state and, if carefully preserved and coloured, show the details of their structure with great clearness. Under the new system of classification, moreover, they are eminently suitable subjects with which to begin the study of the endoparasitic Trematodes.[1] Should these varieties be unobtainable, the small transparent Trematodes found in the intestine of frogs, and which are easily come by, should be used in their place. Other species inhabit the frog, which, though useful, are rather more difficult subjects for experiment. They are found in the oral cavity, generally under the tongue; in the lungs, where they live in company with a Nematode, *Rhabdonema nigrovenosum;* and in the urinary bladder, where the monogenetic Trematode, *Polystomum integerrimum*,[2] is also to be found (fig. 51).

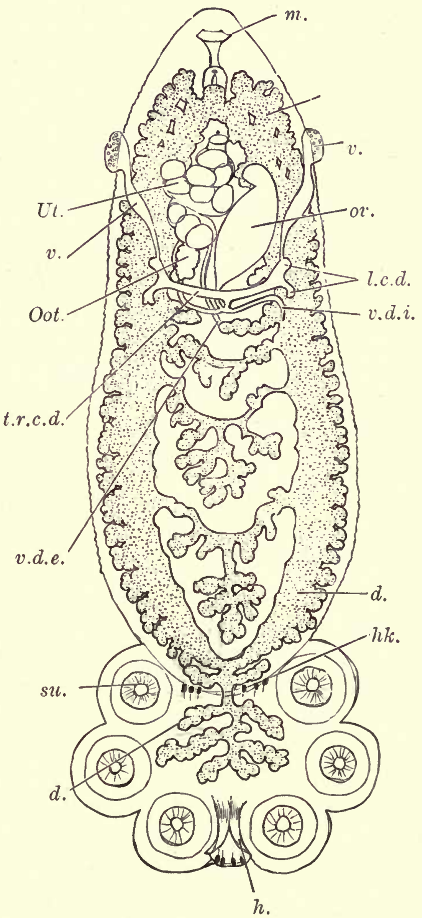

FIG. 51.—*Polystomum integerrimum.* Magnified. *d.*, Intestine. *h.*, Large, *hk.*, small, hooks of the sucking disc. *l.c.d.*, Openings of the yolk-glands (not shown). *m.*, Mouth. *Oot.*, ootype. *ov.*, Ovary. *su.*, Suckers of the attachment disc. *t.r.c.d.*, Transverse yolk-duct. *Ut.*, Uterus containing eggs. *v.*, Mouth of the vagina. *v.d.e.*, Vas deferens. *v.d.i.*, Ductus vitello-intestinalis. (After Zeller.)

After their removal from the frog, the Trematodes should be put into some indifferent fluid upon a glass slide, and covered with a cover-glass of sufficient weight to exercise a slight pressure upon the objects. The movements of the parasites at first make them difficult of observation, but after an hour or so these will cease and the structural details of the organs may be studied. The excretory

[1] The digenetic Trematodes were for a long time arranged under three headings, *Monostomum*, *Distomum*, and *Amphistomum*, classification being based upon the number and position of the suckers. These three genera are now regarded as families, and are subdivided into numerous small classes, the basis of classification, in this instance, being the arrangement and structure of the reproductive organs.

[2] The oral cavity harbours *Distomum ovocaudatum*, now *Halipegus ovocaudatus;* three varieties are found in the lungs, *Distomum cylindraceum*, now classed with

system, which, with the exception of the bladder, is usually empty and for that reason indistinguishable, will now fill and will be clearly seen.

Six Distomes and one Amphistome are found in the intestine of our native frogs and toads. The Amphistome inhabits the terminal portion of the gut, and is distinguished from the Distomes by its sucker, which is placed at the hinder end of the somewhat barrel-shaped body. The sucking organ of the Distomes, on the other hand, is placed in the middle line upon the ventral surface of the body, and has no relationship whatever to the excretory system. The Amphistome is *Diplodiscus subclavatus.*

The six Distomes inhabit the small intestines, and of these one, *Distomum turgidum* = *Brandesia turgida*, lives within the intestinal wall at the commencement of the duodenum. It is enclosed in a cyst nearly as large as a pea, which projects beyond the outer surface of the bowel-wall, and from which the parasite must be removed for the purpose of examination. The five other varieties lie upon the intestinal mucosa and are somewhat difficult of detection, on account of their transparency and their small size. They should be sought with a magnifying glass among the folds of the mucosa; or the mucosa may be scraped off, diluted with normal saline, spread out upon a glass slide, and examined under a low-power microscope. The parasites will be recognized by their movements, and may be picked out with the spatula and put upon glass-slides.

These five Distomidæ are distinguished from one another by the position of the genital opening. In two varieties it is placed in the middle of the ventral surface, just in front of the sucker; in the other three it is situated at the margin of the body. The stomach-tubes will be seen to vary in length, and they may be either long or short in species of either group. When "long" they will extend backwards for a considerable distance beyond the ventral sucker. The parasite which has the genital opening upon the ventral surface of the body, together with short stomach-tubes which do not reach as far as the ventral sucker, is *Brachycœlium crassicolle* = *Distomum crassicolle* (fig. 52); while that with long stomach-tubes is *D. endolobum* = *Opisthioglyphe endoloba* (fig. 53). The two species, which are now recognized as belonging to separate families, differ from one another in certain other respects. The testes, in *crassicolle,* are placed symmetrically at the margin of the body close behind the ventral sucker, while in *endoloba,* they are placed one behind the other in the middle of the body and at some distance from the

Haplometra, and two species of the genus *Pneumoneces* (*Pneumoneces variegatus* and *P. similis*). In addition to the Polystomide already mentioned, the urinary bladder frequently harbours *Distomum cygnoides,* now called *Gorgodera cygnoides* and *Gorgoderina vitelliloba,* though this is of rarer occurrence.

ventral sucker. There is also considerable difference in the position of the ovary; in the shape, position, and length of the yolk glands; in the course of the uterus; and in the form and size of the eggs. There are other minor points of difference which will readily be seen upon examination.

Of the three varieties with the genital opening at the margin of the body, one, *Distomum clavigerum* = *Pleurogenes claviger* (fig. 54), possesses long stomach-tubes and has the testes arranged symmetrically at the hinder end of the body. The other two are furnished with

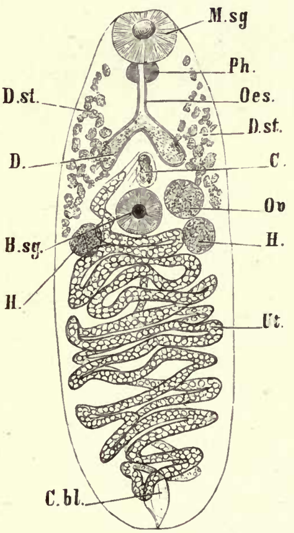

FIG. 52.—*Brachycœlium crassicolle* (Rud.). Slightly magnified. *B.sg.*, Ventral sucker. *C.*, Cirrhus pouch. *C.bl.*, Excretory bladder. *D.*, Intestine. *D.st.*, Yolk-gland. *H.*, Testes. *M.sg.*, Oral sucker. *Oes.*, Œsophagus. *Ov.*, Ovary. *Ph.*, Pharynx. *Ut.*, Uterus containing eggs.

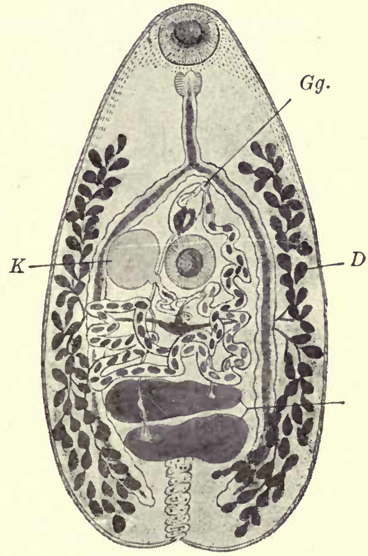

FIG. 53. — *Opisthioglyphe endoloba* (Dy.). 47 : 1. (After Looss.) The lettering is the same as in Fig. 51. *Gg.*, Genital opening. *K.*, Ovary.

short stomach-tubes, and may be distinguished from one another by the position of the testes and ovary. When the ovary is behind the testes, the parasite is *Prosotocus confusus* (fig. 56); when the testes are behind the ovary it is *Pleurogenes medians* (fig. 55).[1]

The preservation of whole specimens presents no difficulty. The parasites are fixed with Hofer's mixture, rinsed in alcohol, stained with alum-carmine, washed out in water, dehydrated by means of the alcohol stages, cleared in creosote, and mounted in balsam.

[1] For further details the student is referred to A. Looss, "Die Distomen unserer Fische und Frösche," Stuttgart, 1894. This work describes in detail all the varieties parasitic in indigenous fish and frogs, and is furnished with excellent diagrams.

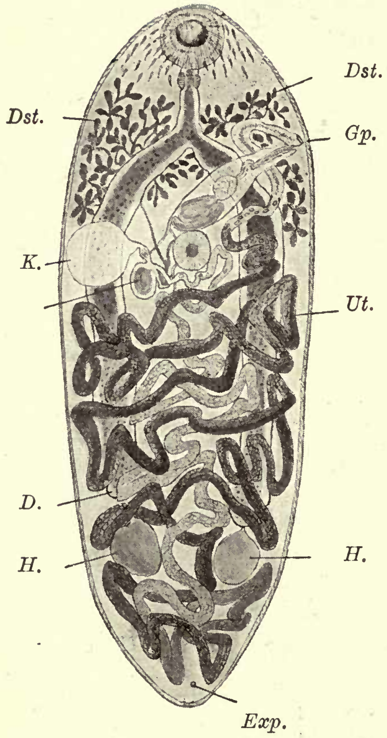

FIG. 54.—*Pleurogenes claviger* (Rud.). 47 : 1. (After Looss.) The lettering is the same as in figs. 52 and 53. *Exp.*, excretory opening.

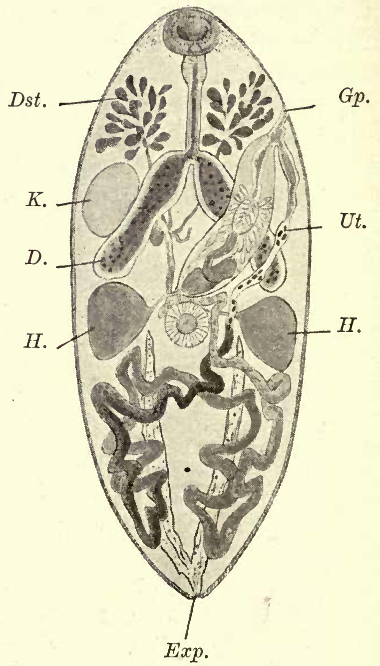

FIG. 55.—*Pleurogenes medians* (Olss.). 47 : 1. (After Looss.) The lettering is the same as in figs. 51 and 54.

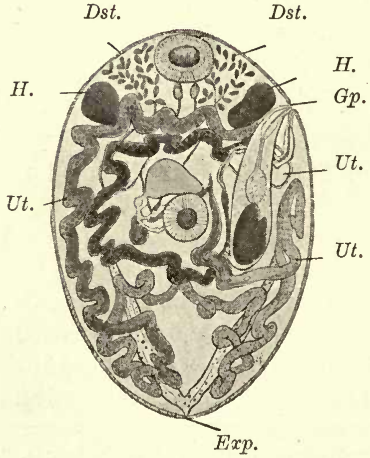

FIG. 56.—*Prosotocus confusus* (Looss). 47 : 1. (After Looss.)

Whole specimens of *Opisthorchis felineus* and *Dicrocœlium lanceatum* are prepared in a similar manner. The comparison of these with the Trematodes found in frogs is very interesting because, though similar in size and form, they differ very materially in their anatomy (figs. 57 and 58).

The lancet-fluke has a long œsophagus and short stomach-tubes, and its genital opening lies close to the ventral sucker, immediately behind the spot where the stomach-tubes fork. In the fluke of the cat, on the other hand, the genital opening is some distance from this spot. The sexual glands (the testes and ovary) of the lancet-fluke are behind the ventral sucker; the large, slightly lobated testes being situated in front of the smaller ovary, which lies a little to the right. In the fluke of the cat, the sexual glands are at the hinder end of the body and, in this case, the ovary, which is small, is

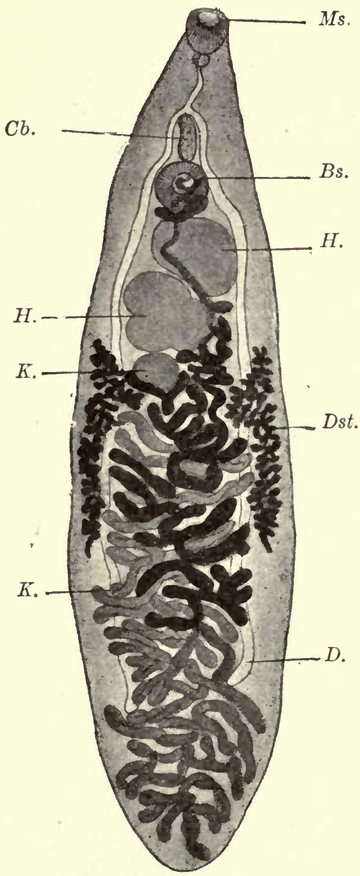

FIG. 57.—*Dicrocœlium lanceolatum*, St. et Hass. 15:1. *Bs.*, Ventral sucker. *Cb.*, Cirrhus pouch. *D.*, Stomach-tubes. *Dst.*, Yolk-gland. *H.*, Testes. *K.*, Ovary. *Ms.*, Oral sucker.

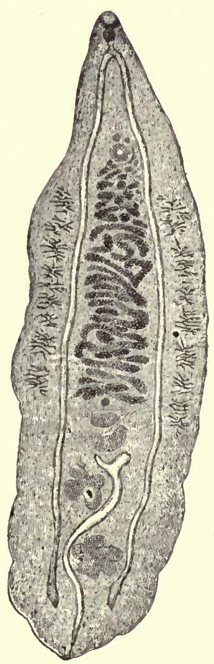

FIG. 58.—*Opisthorchis felineus* (Riv.). 10:1.

placed diagonally in the middle line in front of the testes. The testes, in this parasite, are deeply indented, the anterior gland invariably showing four, the posterior gland five, lobes. The excretory bladder, which runs in an S-shape between the testes, is nearly always distended in *O. felineus*. In the lancet-fluke it is rarely seen, as it collapses in the fixed and coloured specimen. When visible, however, it appears as a straight tube running from front to back. In both species, the position of the yolk-glands is exterior to that of the stomach-tubes, and in the fluke of the cat the yolk-glands are both absolutely and relatively longer than in the lancet-fluke. In the fluke of the cat they extend, rather across than lengthwise of the body, and are united in groups which are only visible in specimens in which contraction is slight. In the lancet-fluke, the follicles of the yolk-glands are rounded and they are not arranged in groups. The course of the uterus is not the same in both species. In the fluke of the cat it is restricted to the field bounded by the stomach-tubes; it runs across this field in folds, which extend forwards from the ovary to the ventral sucker, leaving the hinder third of the body space entirely free. In the lancet-fluke, the uterus commences in the neighbourhood of the ovary and runs in close transverse folds (which occasionally extend beyond the central field) backwards, until it reaches the hinder edge of the body. Here it makes a turn towards the front, throwing out shorter folds towards the sides, until it reaches a position between the ovary and the posterior testis, whence it passes between the testes and, after forming a few more folds in the neighbourhood of the ventral sucker, it finally reaches the surface of the body close to the cirrhus pouch. If we imagine the uterus free from folds, it would run directly from back to front in *O. felineus*, while in *D. lanceatum* it would take a U-shaped course, the one branch (called "descending") extending in a backward direction until it merges into the "ascending" branch, which runs forward to the genital opening. The two branches are largely distinguishable by the difference in colour of the eggs which they contain. The descending portion of the uterus contains the young eggs, which are a light brown in colour and which become gradually darker until, in the ascending branch, they are a brown-black. The difference in shape between the eggs of the two species should be carefully noted (figs. 32, 33). Another point of dissimilarity lies in the nature of the male sexual organs. *D. lanceatum* possesses a distinct organ of copulation, the cirrhus with the cirrhus pouch; while in *O. felineus* this function is fulfilled by the terminal portion of the vas deferens, which, as it is usually filled with spermatozoa, is easily made out close to the ventral sucker.

The points of difference which have been described between the lancet-fluke and the fluke of a cat, may be taken as characteristic of

the manner in which the two families, Opisthorchis and Dicrocœlium, differ from one another. Other species belonging to these families, but proceeding from other hosts, differ in several minor particulars, of which space does not permit a description here.

The liver-fluke (*Fasciola hepatica*) should first be examined in the fresh state, living material being easily obtained from abattoirs. It grows to a length of about 30 mm., and is found in the gall-ducts of sheep and cattle. In infected animals, the vessel walls become thickened and encrusted, and they are frequently dilated to such an extent that the vessels stand out above the surface of the liver. To obtain the flukes, the gall-ducts should be split lengthwise, when cystic pouchings containing flukes will frequently be found.

The flukes should be washed in normal saline, laid upon a large glass slide and carefully stretched with the finger, as they frequently contract when exposed to a temperature lower than that of the body. In appearance, the fluke is a largish, tongue-shaped body, which is prolonged anteriorly into a small, flattened, cone-shaped head process. The difference between the dorsal and ventral aspects should be carefully noted, the ventral surface being recognized by the sucker, which has a diameter of 1·5 to 1·6 mm. and is situated in the middle line at the point where the head-cone widens out into the body. Close to it is the genital opening, from which the long curved cirrhus frequently projects. The scales, by which the anterior portion of both aspects is covered and which project slightly beyond the cuticular layer, are less easy of detection. They may be seen, however, if the parasite is taken up on the finger and, after carefully drying with blotting paper, examined with a strong glass.

If a live fluke is pressed between two glass slides and held, either up to the light or over a white background, a good deal of the internal structure will be seen with a magnifying glass or low-power microscope, or may even be made out with the naked eye. It frequently happens that the stomach-tubes are filled, either entirely or in part, with a dark brown fluid, which acts as a sort of natural injection in showing the the course of the digestive organs. This fluid is blood which has undergone certain changes. The oral sucker will be found at the tip of the head-cone; behind it, in the middle line, is the pharynx, immediately beyond which the œsophageal tube divides. At the level of the ventral sucker, the two branches of the stomach-tube diverge somewhat, though they almost immediately again approach the middle line, and this position is maintained through the rest of their course. The tubes in front of the ventral sucker are furnished with four to five slightly forked appendages, which extend forward and towards the sides. There are similar processes beyond the ventral sucker; these are, however, much longer and divide up into numerous ramifications.

Upon the inner aspects of the tubes these processes take the form of small pouchings (fig. 59).

When the specimen is held against the light, the sides of the tongue-shaped body will appear to be granulated. These granular markings are the yolk-glands, which extend backwards along each side until they meet at the posterior tip of the body, leaving a transparent and lighter central field of the same shape as the body of the fluke.

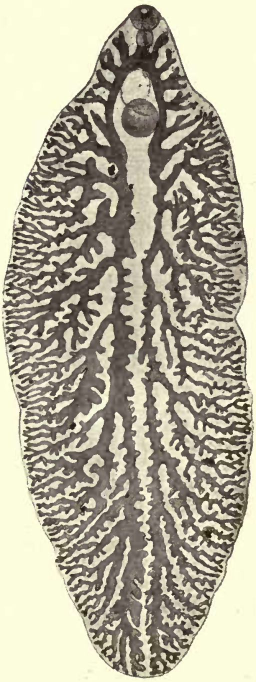

FIG. 59.—*Fasciola hepatica*, L. 5 : 1. Young parasite without organs of reproduction.

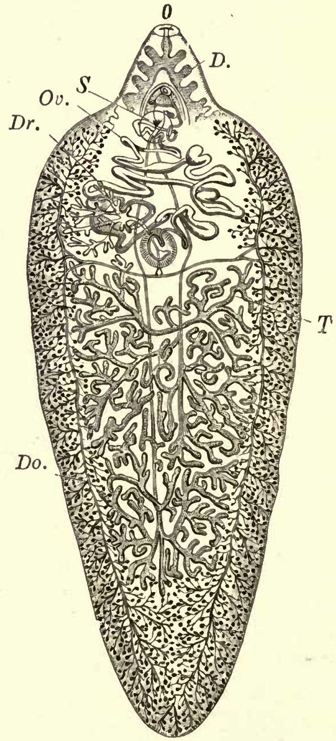

FIG. 60.—*Fasciola hepatica*, L. Slightly magnified. *D.*, Commencement of the intestine. *Do.*, Yolk-gland. *Dr.*, Ovary. *O.*, Oral sucker. *Ov.*, Uterus. *S.*, Ventral sucker. *T.*, Testes. (After Claus.)

The hinder and larger portion of this field is occupied by the testes, which are very much ramified, and are less easy of detection than the yolk-glands. The uterus will, however, be readily seen on account of the opaque eggs which it contains, many of which are of a brownish colour. Its folds lie across the body in front of the testes, and may be traced to the genital opening. It is frequently possible to see eggs in process of extrusion from the mouth of the uterus. Close to the

terminal portion of the uterus, between the ventral sucker and the bifurcation point of the stomach-tubes, a whitish, crooked body is seen. This is the cirrhus pouch, and in dead specimens a portion of the cirrhus is usually extruded from it. The boundary between the field occupied by the uterus and that occupied by the testes is formed by the yolk-ducts, which, when filled with yolk-cells, appear as white threads running transversely of the body. The longitudinal yolk-ducts, which occur in the neighbourhood of the yolk-glands and run parallel to the margin of the body, will also appear as white threads if they contain yolk-cells. At a point in the middle line, the transverse yolk-ducts combine to form a short irregular tube, running towards the head end of the body. This is the yolk receptacle, and in front of it is the shell-gland ("Mehlis' body"), which appears as a whitish disc, about 1·5 mm. in diameter, and is composed of numerous pear-shaped cells arranged radially.

The details of the internal structure will not be seen with equal clearness in every fluke, but by examining several specimens the student will find that he is able to identify all the organs described above. Owing to its position, the ovary is rarely distinguishable in the living object. It is a ramified body, lying in front of the right horizontal yolk-duct, and is hidden partly by the folds of the uterus and partly by the follicles of the yolk-gland.

The distinguishing features of the liver-fluke with regard to form, armature and position of the suckers and of the genital opening, course of the uterus, pronounced development of the yolk-glands, ramification of the intestine, testes and ovary, and the exact duplication of the internal organs on either side of the body, may be taken as typical of the genus *Fasciola* as it is at present defined. These characteristics should be compared with those of *Dicrocœlium* and *Opisthorchis*, as well as with those of the genera of which the intestinal Trematodes of frogs are typical examples.

The stomach-tubes and excretory system of fresh flukes may be injected with Prussian blue or other cold substance. The best instrument for this purpose is a glass tube drawn out at one end to a fine point, which, when filled with colour, is inserted into the oral sucker and pressed down into the pharynx. The mass is expelled by means of an india-rubber ball attached to the large end of the tube, or by blowing down it until the organs are filled. The beginner is very liable to push the point of the glass tube too far down into the pharynx and pierce the œsophageal wall. In this case the stomach-tubes will remain empty, but the excretory system will, as a rule, become filled. The excretory system may always be filled by pricking the body surface at any spot, though this method may give rise to more or less extensive extravasation. The better plan is to introduce

the glass tube into either the main excretory duct, which lies in the middle of the hinder half of the body; or into the excretory aperture, which is situated in the centre of the posterior margin. In either case, there is considerable danger of piercing the thin wall of the excretory duct, though the point is of no great importance, as a certain portion of the system is bound to become filled.

Objects which have been successfully injected should be stretched with the finger, fixed in alcohol between two glass slides, cleared, and mounted in balsam.

The organs containing yolk-cells (yolk-glands, yolk-ducts, and uterus) may be made to show up very distinctly by laying fresh flukes in Müller's mixture between two glass slides, and allowing them to soak until the yolk becomes brown. As soon as the parasite is fixed, the upper glass slide should be removed, either for a time or altogether, in order to allow the fixing fluid to act upon the surface of the body. The specimens are rinsed in running water, or in water that is frequently changed, dehydrated by means of the alcohol stages up to 90 per cent. alcohol, cleared in creosote, and mounted in balsam.

The genital organs will be best seen if the worm is well pressed between two glass slides and treated with Hofer's mixture. The upper glass slide must be lifted from time to time, in order that the fixing fluid may have access to at least one of the body surfaces. The further treatment is carried out by the method described on pp. 99-100. The specimens must be very carefully dehydrated and should be stained with alum-carmine. Over-staining is counteracted by twelve to twenty-four hours' soaking with water, followed by dehydration, clearing, and mounting in balsam.

Specimens intended for section cutting should be laid upon a glass slide, stretched with the finger, and fixed by pouring hot sublimate or other fixing fluid over them. They should be put through the alcohol stages and then divided transversely at the level of the transverse yolk-ducts. The two portions should be stained through with picro-carmine — this will take about twenty-four hours — the superfluous colour being afterwards rinsed out in water. They are then dehydrated by carefully graduated alcohol stages to absolute alcohol, cleared in turpentine or xylol (very inflammable), and embedded in paraffin. The finished specimens should be attached to the glass with collodion clove oil; but if the sections are to be further coloured after cutting, they should be attached to small cover-glasses by means of water, which is allowed to evaporate upon a thermostat.

To obtain a minute knowledge of the structure of the liver-fluke, transverse and longitudinal sections in series should be prepared from several individuals. The beginner will find it sufficient, however, to prepare single sections from certain parts. Such are: transverse

sections from the posterior portion of the body in the neighbourhood of the testes (fig. 61); from the hinder end of the anterior portion in the neighbourhood of the ovary; and one or more sagittal sections taken in the median plane from the anterior body-half, showing the oral sucker, pharynx, cirrhus pouch, and ventral sucker. One worm divided transversely into two will supply these more important sections, which should be carefully compared. The cuticle with its armature should be noted, as well as the muscular structure and the parenchyma. The student should then seek to identify the special organs. In transverse sections taken at the level of the testes, the stomach-tubes will be very noticeable, their side branches, which will

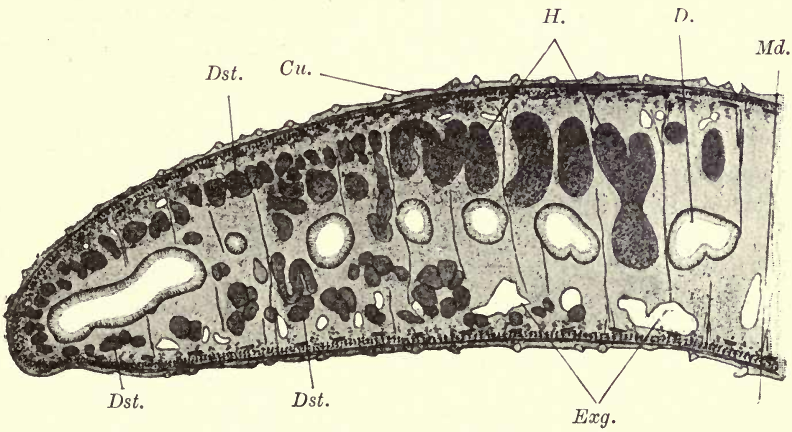

FIG. 61.—Half of a transverse section from *Fasciola hepatica*, L., taken at the level of the testes. 25 : 1. *Cu.*, Cuticle with armature; below it are the ring, longitudinal, and transverse muscles. *D.*, Stomach-tubes; the hollow spaces are the blind branches of the intestine which have been severed either diagonally or transversely. *Dst.*, Yolk-gland follicles. *Exg.*, Excretory vessels. *H.*, Branches of testes. *Md.*, Median line.

have been cut through either transversely or diagonally, appearing as round or oblong cavities lined with a single layer of epithelium. At both the dorsal and ventral margins, numerous yolk-gland follicles will be seen. Those at the ventral edge approach nearer to the middle line than those of the dorsal edge; they do not, however, touch it. On the dorsal side, the space between the yolk-gland follicles and the middle line is occupied by the larger branches of the testes, which also extend into the central field. A transverse section taken at the level of the ovary will show parts of this organ upon one side; towards the centre, the uterus folds containing eggs will be seen in section. If the eggs have taken the stain well, the details of their structure—namely, the lidded shell, germ-cell, and numerous yolk-cells—will be apparent. In sections taken a little lower down, the shell-gland will also be seen. Longitudinal sections through the head

end, close to the middle line, show the relationship of the different parts of the sucking apparatus (mouth, pharynx and ventral sucker) to one another; they will also include the cirrhus pouch and, frequently, the cirrhus (fig. 62).

Two species of Paramphistomide (*Gastrodiscus hominis* and *Cladorchis watsoni*) occur in man, and it is expedient for this reason that the student should make himself acquainted with the structural peculiarities of the Paramphistomatidæ of mammals. The best subject is *Paramphistomum cervi,* frequently found in the paunch of oxen.

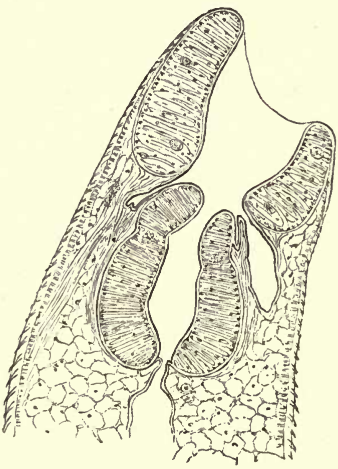

FIG. 62.—*Fasciola hepatica,* L. Longitudinal section in the median line through the anterior portion of the head process, showing mouth, pharyngeal sacs, pharynx and œsophagus. Magnified. (After Leuckart.)

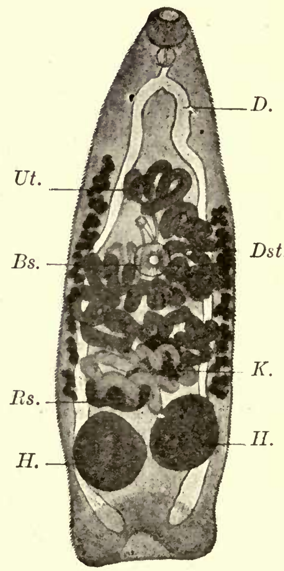

FIG. 63.—*Metorchis truncatus* (Rud.)., 25 : 1. *Bs.*, Ventral sucker. *D.*, Stomach-tubes. *Dst.*, Yolk-glands. *H.*, Testes. *K.*, Ovary. *Rs.*, Receptaculum seminis. *Ut.*, Uterus.

Specimens should be killed in Müller's mixture, alcohol, or sublimate, and cleared in creosote. A representative of the Schistomatidæ will not be found in these latitudes, although *Schistosomum hæmatobium* occurs in man with some frequency in Egypt, and another species of the same family is found in cattle in Southern Europe.

The varieties of Trematode parasitic to indigenous domestic mammals are not very numerous. The liver-fluke is found in the gall-ducts of sheep and, less frequently, in cattle, goats, horses, asses, and rabbits. The lancet-fluke, though rarer, may occur alone or in company with the liver-fluke, and occasionally "wanders" to the

lungs, especially in cattle. In the gall-ducts of cats and dogs, the fluke of the cat (*Opisthorchis felineus*) is found, together with a very small variety, *Metorchis truncatus* (Rud.) (fig. 63), which does not exceed 2 mm. in length. Cats also harbour *M. albidus* (Braun), the length of which varies from 2·5 to 3·5 mm.[1] The liver-fluke is pathogenic to sheep, the cat-fluke to cats and dogs.

Paramphistomum cervi (= *Amphistomum conicum*, Rud.), occurs in the paunch of cattle, sheep, and goats. The worm is about 1 cm. in length, and is attached to the mucous membrane between the papillæ by its hinder end. It is prepared by the methods described above.

The Trematode fauna of domestic birds is somewhat more numerous. The following Echinostomes occur in the intestine of geese, ducks, and hens: *Echinostomum conoideum* (Bloch) = *Distomum oxycephalum*, Rud.; *E. revolutum* (Fröl.) = *D. echinatum*, Rud.; and *E. recurvatum* (v. Lstw.). These varieties are characterized by the possession of a collar, studded with hooks, at the anterior end. *Prosthogonimus pellucidus*, which is found in the terminal portion of the intestine of hens, is characterized by the position of the genital opening, which is situated anteriorly, close to the oral sucker. This parasite frequently penetrates to the oviduct of its host, becomes enclosed in the eggs, and may be found in them after they are deposited.[2] Of the Monostomatidæ, *Notocotyle verrucosa* (Fröl.), which grows to a length of 5 to 6 mm., is frequently met with in the cæcum of geese and ducks; while grazing geese harbour *Monostomum arcuatum* (Brds.), a parasite 20 mm. in length, which is found in the cellula infraorbitalis.

The Development of Digenetic Trematodes.—The development of the ova, and of the miracidia from the ova, has been already described. Where the artificial infection of molluscs is not possible, indigenous fresh-water snails and mussels should be examined during the summer months, for cercariæ, rediæ and sporocysts. The mollusc most frequently affected is *Limnæus stagnalis*, which lives among water plants in stagnant ponds and glides over the surface of the water with dependant body. There are other varieties of the same or of allied genera (*Planorbis, Paludina, Bithynia*, &c.), many of which live in quiet eddies of running water, where sporocysts are also to be found. The snails should be put alive into small glass vessels with sufficient water; the vessels should be covered and allowed to stand. If infected individuals are among the snails, the cercariæ will emerge and will be seen macroscopically, after about twenty-four

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[1] Braun, *Centralb. f. Bakt. und Paras.*, vol. xiv, 1893, p. 381.

[2] Braun, *Centralb. f. Bakt., Paras. und Inf.*, part 1, vol. xxix, 1901, p. 12; and *Zool. Jahrb. Syst.*, vol. xvi, 1902, p. 67.

hours, as small whitish bodies in the water. Their movements are somewhat rapid and they remain at first in the neighbourhood of their host. They should be lifted out of the water with a pipette and put into a watch-glass or hollow glass slide with a little water. If they are now examined with a low-power lens, the peculiar nature of their movements will seen. The anterior portion of the body is drawn up into a ball (fig. 64), while the tail moves so rapidly as to be scarcely discernible. After a varying period of time, this movement slackens and the animalcule rests, or, with the aid of the suckers at the anterior part of the body, crawls about for a time at the bottom of the vessel before recommencing its swimming movements. The structure of the cercariæ should be studied in living specimens under the microscope. This is done by putting them in a little water on to a glass slide, and using supports to prevent crushing by the cover-glass. The supports (strips of paper) should be of sufficient thickness to pin the parasite between the cover-glass and slide and thus prevent swimming, but not crawling, movements. The cercariæ are sufficiently transparent to allow of the organs being clearly seen, and especially is this the case just before they die. Many varieties have a boring organ within the oral sucker, and occasionally, though more rarely, eye-spots are seen.

FIG. 64.—Cercariæ of *Echinostomum* sp. (from *Limnæus stagnalis*). 25 : 1.

Cercariæ are easily fixed and coloured ; they should be mounted in balsam. Large numbers should be treated in watch-glasses, single specimens on glass slides, the method being the same in both cases.

The sporocysts and the rediæ from which the cercariæ are evolved, are obtained by breaking a snail, which is known to be infected, out of the shell, when the sporocysts will be seen as long yellowish bodies underneath the mantle. Sometimes they occur in the liver or in the sexual glands, and here also their yellow colour renders them easily recognizable. The material should be diluted with snail's blood or with normal saline, which should be spread out upon a glass slide and examined with a low-power lens. There is very little to observe in the sporocysts themselves ; they are quite simple structures, entirely filled with cercariæ in various developmental stages. The

rediæ are slightly more complex. They are characterized by the possession of an alimentary canal and, in the greater number of cases, it is possible to make out the pharynx, together with a portion of the single straight intestinal tube (fig. 65).

The pressure exerted by the cover-glass, and by manipulating and spreading the material upon the slide, will set free many cercariæ in various stages of development. These should be carefully studied. It is more expedient, however, to remove the sporocysts without subjecting them to pressure, fix them in hot sublimate and, after staining, cut them into longitudinal sections.

The sporocysts of certain varieties are branched; such are those which occur in the snail (*Succinea amphibia*), and in certain mussels (*Anodonta, Unio*). In both these instances, the cercariæ show considerable divergence from the type described above. Those which develop from the sporocysts found in *Succinea* are not provided with a tail and do not emerge from the sporocyst, but encyst in its blind branches.[1] The cercariæ which are found in the mussel varieties, on the other hand, emerge from the sporocyst and are provided with a tail which is split along its entire length.[2] Cercariæ with partially split tails are occasionally found in Limnæidæ, while cercariæ with blunt tails are found in land-snails.

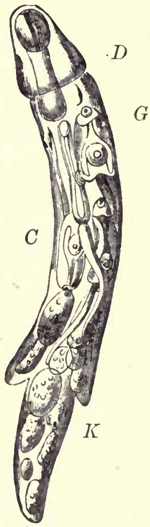

Fig. 65. — Redia of *Echinostomum* sp. (from a fresh-water snail). Magnified. (After Leuckart.)

Instructive material is also furnished by encysted Trematodes, some of which, while in this stage, become sexually mature and form eggs. They are found in widely different organs in hosts of many species, both vertebrate and invertebrate.[3]

(2) Cestodes (Tapeworms).

Of the tapeworms parasitic in man a small number only have any very wide distribution, and these are principally met with in Central

[1] Infected *Succinea* may be recognized by the tentacles, which become very swollen and through the thin walls of which a brightly coloured cylindrical structure, which is actively motile, may be seen. This structure is the mature blind end of the sporocyst. See Zeller, *Z. f. wiss. Zool.*, xxiv, 1874; and G. A. Heckert, *Bibl. zool.*, vol. iv, 1889; also Bronn's "Kl. u. Ordn. d. Thierr."

[2] This cercaria is known as *Bucephalus* and is a developmental stage of *Gasterostomum*, which inhabits the intestine of predatory fish. See Ziegler, *Z. f. wiss. Zool.*, vol. xxxix, 1883; and Bronn's "Kl. u. Ordn. d. Thierr." vol. iv, 1 a.

[3] O. v. Linstow, "Compendium der Helminthologie," Hanover, 1878; Nachtrag, Hanover, 1889.

Europe. They are *Tænia saginata*, *T. solium*, and *Dibothriocephalus latus*. The last-named is found in certain districts only, where it is also parasitic in the dog, the cat, and the fox. The two Tænia varieties are confined solely to man and they may be extra-European in their occurrence. To these three species *Hymenolepis nana* must be added; it is native to the districts bordering the Mediterranean, though it is also met with farther north. It is a specific parasite of man. All other European tapeworms, and probably also those which occur outside Europe, are only occasional habitants of man, their normal hosts being mammalians of other genera and in one (doubtful ?) case, of birds. To these belong *Dipylidium caninum* of dogs and cats; *H. diminuta* of rats and mice, which has been encountered only in the South of Europe and outside it; and *H. lanceolata* of geese and other fowl, which was upon one occasion found in a man at Breslau. Man may also harbour the Cysticercoid stages of certain worms. To some of these he is also the definitive host (*T. solium*, *T. saginata*); others, again, only attain maturity in other mammalians (*T. echinococcus* of dogs); while, in a certain proportion of cases, encountered only in Eastern Asia, both the adult parasite and the host which harbours it, are unknown.

The varieties most easily obtainable in Central Europe are: *T. saginata* of man; *Dipylidium caninum* of dogs and cats; *H. diminuta* of rats and mice; and *H. lanceolata* of geese. *T. solium*, which was formerly very prevalent in Northern Germany, has now become scarce,[1] while *Dibothriocephalus latus* is very limited in its distribution, being found only in the coast districts of Northern Germany (especially towards the east), in the neighbourhood around Starnberg, and in Switzerland. Of the Cysticercoids observed in man, those most easily obtainable are: *Cysticercus cellulosæ*, of the *T. solium* of swine; *C. bovis*, of the *T. saginata* of cattle; and *Echinococcus veterinorum*, of the *T. echinococcus* of cattle, swine and sheep.

Cestodes should be examined as follows: A whole worm should be washed in tepid normal saline and then put into a shallow glass vessel of sufficient size, together with clean normal saline, and placed upon a dark background. Preserved specimens are taken out of the vessels in which they have been stored and are put, with the preserving fluid, into shallow bowls or on to plates. The worm will be found to consist of a varying number of segments or proglottides, which increase in size and alter in shape towards the posterior end. At the anterior end is

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[1] A good substitute for demonstration purposes is *T. crassicollis* of cats or, better still, one of the larger varieties (*T. marginata*, *T. serrata*, *T. cœnurus*) from dogs. Cestodes which have been expelled by means of anthelminthics from man, are usually useless for histological purposes.

the neck, a homogeneous portion of varying length, which terminates in the head. In all Tæniidæ, the head is furnished with four suckers arranged radially (only to be made out in small species with a strong lens or microscope), and, in the greater number, it is crowned with a rostellum. This rostellum, which is frequently more or less invaginated, varies considerably in shape and is furnished with hooks. In Bothriocephalus, the head is elongated and possesses two longitudinal furrows of varying depth, which are continued into the suctorial grooves. Owing to the fact that the dorso-ventral diameter is less than the transverse diameter, the head usually lies over on its side, and for this reason the grooves appear to be placed laterally.

The proglottides also show considerable divergence of form. Those at the head end are broader than they are long, and they increase in size and in structural development as they progress towards the tail end. At the lateral margin of the larger Tæniæ a slight eminence, more or less distinctly defined, will be observed. This is the genital papilla, with the genital opening at its summit. In the greater number of species, the papillæ alternate with comparative regularity; in Hymenolepis, however, they are always placed at the edge of the left margin, while in Dipylidium there are two genital openings to each segment, placed one in the centre of either lateral edge.

The genital pore is always furnished with two openings, those of the cirrhus and the vagina, while in Dibothriocephalus there is a third, namely, the uterine opening, from which the eggs are extruded. In the other Tæniidæ there is no uterine opening to the exterior, the eggs being liberated only by the decomposition of the proglottides. The three genital openings of Dibothriocephalus lie close behind one another, in the middle line, upon that flat surface of the body which is usually termed ventral. In well-preserved specimens they may be seen with the magnifying glass, though they are less easily made out in living material.

The proglottides of all tapeworms vary according to their age, which, as they are formed successively from the neck, is in direct ratio to their distance from it. The youngest and narrowest proglottides do not possess anything in the nature of reproductive organs. These begin to appear in somewhat older segments, becoming more developed the farther removed the segments are from the head. Those in which all the sexual organs are fully formed are called "mature."[1] As in most hermaphrodite animals, the male sexual

[1] Proglottides with fully developed male and female organs are described in this work as "mature." For those older proglottides, in which retrograde changes of the sexual glands have already taken place and in which the uterus is filled with the oval the term "ripe" is employed.—Translator.

glands mature first and this maturity is followed by copulation, generally reciprocal. The ripe spermatozoa are stored in the receptaculum seminis, a special enlargement of the vagina, until the female glands are ready to supply their products—the ovary the eggs, the yolk-gland yolk-cells. The fertilized ova pass into the uterus, causing it to unfold, while more or less complete retrograde changes take place in the germ-preparing glands, beginning with the testes. In the oldest ripe proglottides of the larger Tæniæ, all that remains of the sexual organs is the cirrhus-pouch, portions of the vas deferens and vagina, traces of the shell-gland, and the completely developed uterus. The uterus is composed of a main stem running from front to back in the median line, from which are thrown out on both sides a quantity of ramifying branches with blind ends, the number of which varies in different species. The ramifications of the uterus, together with the shape, size, number and arrangement of the hooks on the rostellum, are points of importance in distinguishing between species.

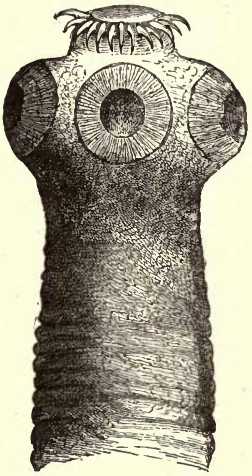

Fig. 66.—Head and neck of *Tænia solium*, L., showing the appearance of the head in the large-hooked varieties of the Tæniæ of mammals. 45 : 1.

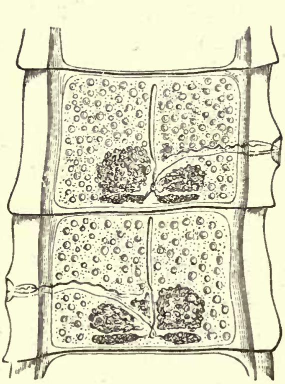

Fig. 67.—Two mature proglottides of *Tænia solium*, showing the arrangement of the sexual organs in the large-hooked Tæniæ of mammals. Slightly magnified.

If a ripe proglottide of one of the larger Tæniæ of man, the dog or the cat, is pressed between two glass-slides and held up to the light, the uterus, which may be seen with the naked eye, will appear as an opaque tree-like structure in a transparent field. The appearance of the uterus in ripe segments of *Dipylidium caninum*, however, is quite otherwise. It is best seen in mature segments which have been carefully stained and coloured, where it has the formation of a net, in the meshes of which the testes are placed. After the entrance of the eggs, the uterus breaks up into parts, each containing several eggs, and a reddish granular mass is secreted, which encloses the eggs and cements them together in masses. After the disappearance of the uterus-wall, these egg-clusters lie free in the parenchyma and this gives to the ripe segments a reddish colour. The egg-clusters are easily obtained by teazing out a ripe joint (fig. 36).

Ripe segments from the hinder middle portion of Dibothriocephalus, if examined in the same manner, reveal other conditions. The proglottide is divided longitudinally into three fields, which differ in colour and in degree of transparency. The two lateral spaces are opaque and yellowish-brown in appearance, owing to the presence near the surface of innumerable little granules—the yolk-gland follicles. The central field is more transparent and contains an apparently branched or rosette-shaped organ, in front of which is a rounded structure of whitish colour. The latter is the cirrhus-pouch, while the rosette-like organ is the uterus, arranged, as in *Fasciola hepatica*, in transverse folds, which are here filled with brownish eggs. The loops are approximated in various degrees, enclosing wider or narrower fields, according to the state of contraction of the segments. End proglottides sometimes have the uterus partially or completely empty. This is owing to the fact that the uterus is furnished with an opening through which the eggs escape, while the sexual glands become atrophied and cease to form eggs.

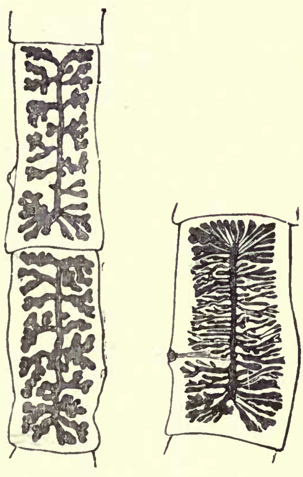

FIG. 68.—Ripe proglottides of *Tænia solium* (left) and *T. saginata* (right), showing the development of the uterus in the large-hooked Tæniæ of mammals. 2:1.

The terminal segments of tapeworms, as is well known, are released, either singly or in numbers, and are conveyed to the exterior in the fæces of their host. If this has not as yet taken place, it is certain that the specimen under examination is comparatively young. In such a case, the terminal proglottides of the greater number of cestode species, if not all, will be smaller in size than in the adult worm, and will be deficient in sexual organs.

The student should be on the watch for abnormalities of structure. In Bothriocephales these are usually due to the interpolation of wedge-shaped joints, though fenestration of single or of successive joints is also frequent, as also the absence or incomplete formation of the line of demarcation between the joints. Duplication of the genital pores, as well as of the genital glands; reversal of the genitals; bifurcation of the segmented body, and duplication of the entire worm, which then assumes a triagonal form, are also malformations which are not infrequently met with.

Before preparing fresh Cestodes for permanent preservation, a ripe proglottide should be detached and teazed out on a glass slide

for the purpose of obtaining the eggs. This manipulation serves also to show the calcareous bodies, which are recognized by their high

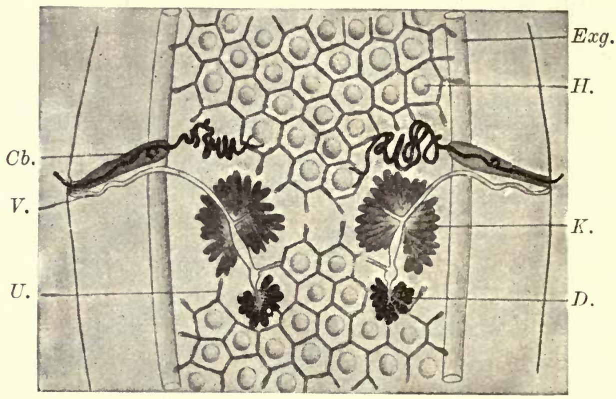

FIG. 69.—Central portion of a mature segment of *Dipylidium caninum*. Magnified. *Cb.*, Cirrhus pouch. *D.*, Yolk-gland. *Exg.*, Excretory vessel. *H.*, Testes. *K.*, Ovary. *U.*, Uterus. *V.*, Vagina. (After Neumann and Railliet.)

refractivity and their concentric stratification. They are situated in the parenchyma and, when present in large numbers, give to the worm its chalk-white appearance. Cestodes in which but few calcareous bodies are present, are yellowish-white in colour and are of greater transparency.

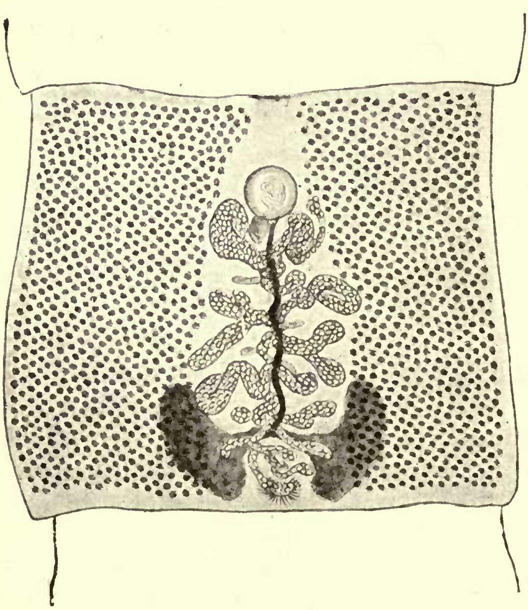

FIG. 70.—Mature proglottide of *Dibothriocephalus latus* (L.). The lateral fields contain the numerous yolk-gland follicles; the central field contains the cirrhus-pouch, uterus, vagina, ovary, and shell-gland. 15 : 1.

Whole preparations should be made of the head, of mature, and of ripe proglottides, and sections of these parts should also be prepared.

The preparation of ripe segments whole offers no difficulty. The fresh proglottide is pressed between two cover-glasses, fixed in alcohol, and treated with alcohol and glycerine, in which it is allowed to

remain until the alcohol has evaporated and the specimen lies in pure glycerine. It is then mounted in glycerine-gelatine. Or the segments may be fixed in alcohol, dehydrated in strong alcohol, cleared in creosote or oil of cloves, and mounted in balsam. Specimens prepared by the second method acquire considerable transparency, but the uterus, unless filled with eggs, is unrecognizable (fig. 68).

Whole preparations of mature segments, if prepared in the right manner, show the entire reproductive apparatus with great distinctness. This does not apply to worms with well-marked muscular structure, such as *T. crassicollis,* though, with this exception, the method may be employed for the varieties which have been mentioned. One or more segments, in a suitable stage of development, should be taken from the anterior middle portion of the worm and killed by the method described on pp. 100-101. After the final alcohol stage, they should be stained with well-diluted ammoniated carmine, or with alum-carmine. They are differentiated—in the first case, with slightly acidulated water, in the second with pure water—and mounted in gelatine or balsam. If the sexual ducts are empty, their openings will not be visible. For this reason it is advisable to prepare a large number of segments, in one or other of which the ducts will eventually be seen (figs. 67, 69, 70).

In the case of Tæniidæ, preparations such as these show not only the genital organs, but also portions of the excretory system, especially the large canals at the lateral margins of the joints and the transverse ducts at the posterior border. The smaller tributary vessels are rarely visible. In Dibothriocephalus the excretory system is net-like in character, and is best seen in the fresh object.

The head is prepared in a similar manner. In the case of the larger and more muscular varieties, however, a good deal of pressure is required before the suckers and rostellum are seen, and the head will become flattened out of shape in the process. The Cysticercoid stages may also be treated by this method; this subject, however, will be dealt later.

For purposes of more minute investigation, series of sections, taken in different planes from mature proglottides, are very instructive. As a general rule, however, single transverse sections through certain zones of mature proglottides from a variety of Tænia and a variety of Bothriocephalus, will be found sufficient. They should be cut at the level of the testes, of the ovary, and of the cirrhus-pouch. The proglottides should first be fixed with hot sublimate and then stained with picro-carmine.

The first point to be studied in the finished section is the structure of the proglottide. A transverse section is ribbon-like in shape, and is

rounded at the lateral edges. It is enclosed in a homogeneous cuticle of moderate thickness, within which, with the aid of a strong glass, is seen a second, thinner, homogeneous layer—the basement membrane. This is intimately associated with a thin muscular structure, composed of transverse and longitudinal fibres, the ends of the longitudinal fibres appearing in the transverse section as small bright discs. Within this structure is the so-called subcuticular layer, composed of spindle-shaped cells arranged radially, which, in the section, have been split along the axis-line. These cells resemble epithelial cells in their arrangement, but they do not constitute a true epithelium. If very thin sections from an uncontracted proglottide are closely examined, it will be seen that the cells are separated from one another by parenchyma, and that they do not all lie at the same level. The parenchyma itself is very difficult of examination, and its structure is differently described by different authors. It fills the entire space enclosed by the subcuticular layer, and in it are embedded the calcareous bodies, the various organs, and the longitudinal muscles. The longitudinal muscles are situated in the peripheral zone, and their development varies in different species. Their inner surface is everywhere bounded by transverse muscles, which enclose the central field. The part of the section within the transverse muscles is called the "middle-layer," while that outside it is called the "cortical layer." By treating the specimen with some acid solution, the carbonate of lime which the calcareous bodies contain will be released, and they will become clear.

In addition to the muscles already described, single fibres running in a dorso-ventral direction, with brush-like ends which reach the basement-membrane, will be seen. These are the parenchyma muscles.

With the exception of the peripheral portions of the cirrhus, cirrhus-pouch, and vagina, all the sexual organs of Tæniidæ are included in the middle layer. In Dibothriocephalus, the follicles of the yolk-gland—which are of considerable size and are equally distributed upon both sides—lie outside the longitudinal muscles and are also included in the cortical layer, which is, moreover, penetrated by the uterine orifice.

Sections of Tæniæ will show the lumens of the excretory canals (fig. 71). Two are placed at each lateral extremity of the middle layer, the external tubes being larger than the internal ones. In the neck and in young proglottides, these tubes are of equal diameter; the two inner ones, however, begin to decline with increasing growth and, in the ripe segments, they have usually completely disappeared. Externally to the larger tubes, on both sides of the middle layer, lie the cut surfaces of the nerve-cords, accompanied, in each case, by two

smaller strands placed dorsally and ventrally of the main cord. They appear as finely dotted discs. In Dibothriocephalus, these nerves lie closer to the middle line, well within the middle layer; while the excretory vessels, the net-like formation of which has already been described, appear as variously-shaped gaps in the parenchyma of the cortical layer. Sections of Dibothriocephalus differ, moreover, from those of the Tænia varieties in the arrangement of the reproductive organs. These will be readily identified, however, if their arrangement has been previously studied in whole specimens.

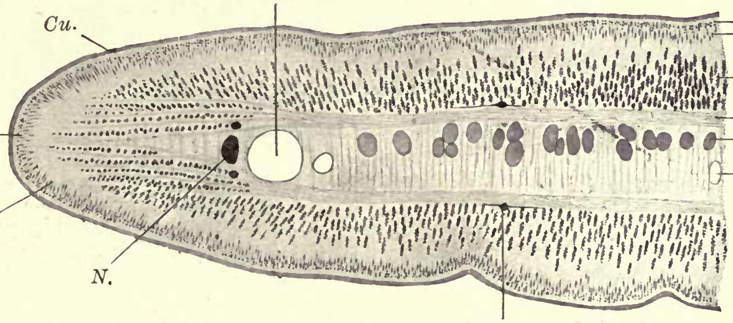

FIG. 71.—Half of a transverse section from a mature proglottide of *Tænia crassicollis*. *Cu.*, Cuticle. *Exg.*, External excretory vessel (to the right of it is the internal excretory vessel). *H.*, Testes. *Lm.*, Longitudinal muscles. *N.*, Nerve-cord with accompanying strands. *Scz.*, Subcuticular cells. *Smn.*, Sub-median nerve-cord. *Trm.*, Transverse muscles. *Ut.*, Uterus. 44 : 1.

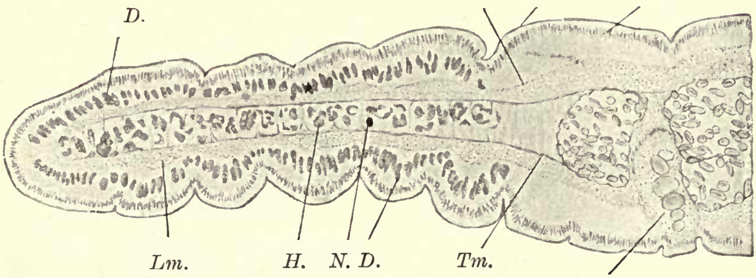

FIG. 72.—Half of a transverse section from a proglottide of *Dibothriocephalus latus*, taken at the level of the cirrhus-pouch, *C.* *Cu.*, Cuticle. *D.*, Yolk-gland follicles. *H.*, Testes. *Lm.*, Longitudinal muscles. *N.*, Nerve-cord. *Sc.*, Subcuticular layer. *Tm.*, Transverse muscles. *Ut.*, Uterus. 30 : 1.

The beginner should not fail to make sections of the head in order to make himself familiar with the structure of the suckers and, in the case of the Tæniidæ, of the rostellum. Sections of the rostellum should be taken transversely. The study of the nervous system of

the head is a more difficult matter and is only to be carried out by means of sections in unbroken series.

Examination of Cysticercoid Stages.

The Cysticercoid stages (*Cysticercus tenuicollis*) of the *Tænia marginata* of dogs, which undergoes development in the omentum of sheep, are readily obtainable from abattoirs. On account of their large size, they are very suitable subjects for a first examination of the structure of Cysticerci. When fresh, the Cysticercus is generally still enclosed in the cyst, derived from the tissues of its host, which must be opened with great care to avoid damaging the inmate. Attached to the body of the Cysticercus is a chalk-white, cone-shaped formation, which projects in a varying degree above the surface of the bladder, and at the free end of which an orifice, into which a bristle may be inserted, is situated. This projection is the head-cone. It is a hollow organ with thick walls which are continued inwards, and it contains the head parts of the future tapeworm (suckers, rostellum), which develops inwards within the hollow. As soon as the Cysticercus is imported into the intestine of the definitive host, namely, the dog, the head-cone becomes completely everted and forms the scolex of the future tapeworm, the bladder at the same time perishing. In *Cysticercus tenuicollis,* as in many other species, this process of evagination frequently begins in the intermediate host, and it may be completed by artificial means in the isolated specimen. The parasite should be held just below the head-cone in the finger and thumb of one hand, while a regular pressure from within outwards is exerted with the fingers upon the head-cone. The head-cone will lengthen and, if the manipulation is repeated several times, it will turn inside out and will hang down as a flat, wrinkled, contractile band upon the surface of the bladder. If pressure is now exerted upon the free end, the head will finally become everted, springing out with a sudden jerk. It will appear as a round body of considerable transparency (owing to the absence of calcareous bodies) at the end of the chalk-white band of the everted head-cone. It is not easy to examine the head in this condition because, as soon as pressure is removed, it is usually again retracted. The head should, therefore, be cut off from the head-cone, the peripheral end being laid upon a glass slide, and pressure should be exerted upon the cut end until the head again appears, when a cover-glass should immediately be placed upon it. It is frequently impossible to extrude the head completely, the rostellum remaining partially inverted. In this case the hooks, with which the edge of its summit is crowned, will have their points directed upwards instead of sideways. Successful specimens may, however, be obtained, and these should be fixed

under the cover-glass in alcohol. They are treated with alcohol and glycerine, and finally enclosed in glycerine-gelatine.

It is a more difficult matter to effect the evagination of the head in the smaller Cysticercoid varieties, such as *C. cellulosæ* of swine, *C. bovis* of cattle, and *C. pisiformis* of rabbits. But in these more transparent varieties, the head is seen lying within the bladder and may be cut out. It should be pressed between two glass-slides and examined with a low power microscope. Good specimens may be converted into either stained or unstained permanent preparations.

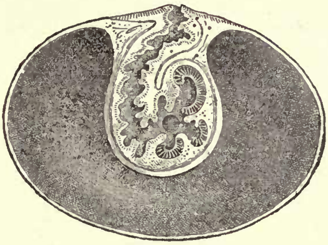

FIG. 73.—Longitudinal section through a Cysticercus. (After Leuckart.)

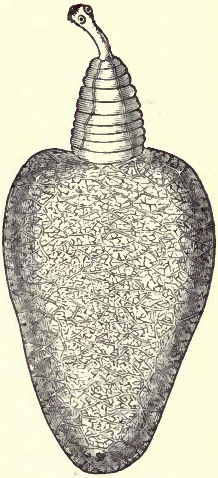

FIG. 74.—*Cysticercus pisiformis*, with completely evaginated head and body. 18 : 1. (After Leuckart.)

In the case of the larger Tæniæ, such as *T. crassicollis*, the rostellum and hooks of which are seen with the naked eye, it is easy to sever the protruded end of the rostellum with a razor, and, after clearing and mounting (glycerine or balsam), to examine it under the microscope.

The Echinococcus is found in the liver and lungs of animals fattened for slaughter, and is the Cysticercoid stage of the small *T. echinococcus*, which inhabits the intestine of the dog. The encysted parasite may grow to the size of an apple, though the cysts found in cattle are frequently sterile. In such cases, the bladder-wall is composed only of the cuticular layers and of the delicate germinal layer attached to their inner surface. In fertile Echinococci, the inner surface of the germinal layer shows numerous small, thin-walled bladders; these are the brood-capsules, from the walls of which numerous scoleces have been derived, and these may be found in all stages of development. These scoleces will be seen if portions of the germinal layer are removed from the cuticle and spread out upon glass-slides, wherever possible in liquid from the cyst (fig. 76). To fix these, portions of germinal layer should be spread out on a

small glass bowl coated with wax, or on a sheet of cork, and hot sublimate or other fixing fluid should be poured over them. They are finished in the usual manner. The form of the scolex and the shape of the hooks should be carefully noted, as these points have a diagnostic significance.

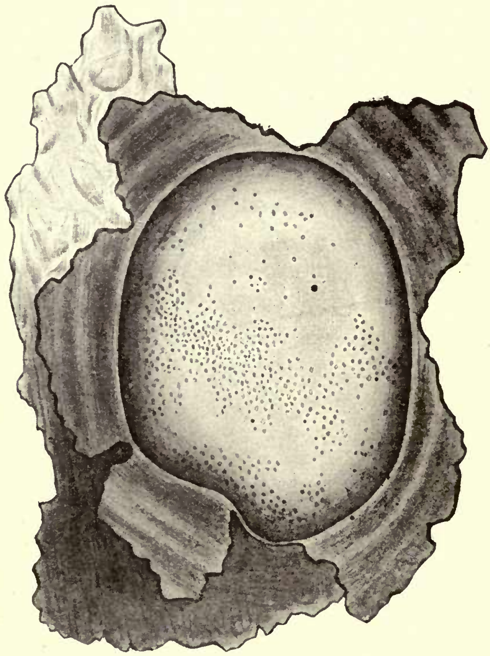

Fig. 75.—*Echinococcus veterinorum.* The cystic membrane surrounding the Echinococcus is opened and turned back in five points. The surface of the parasite is seen with the brood-capsules showing through it. (After Leuckart.) Natural size.

There is a variety of Echinococcus, almost invariably met with in cases of human infection, though rare in cattle, which forms daughter - bladders. The daughter-bladders lie within the mother-bladder, or between it and the surrounding cystic wall. They are structurally similar to the mother-bladder, which is the direct outcome of the oncosphere, and they are frequently sterile. A certain proportion form brood-capsules containing scoleces, while others, again, produce a second generation of daughter (or grand-daughter) bladders, similar in construction to themselves. By introducing fresh Echinococcus heads into the pleural or peritoneal cavities, or under the skin, of rabbits, it has been shown that the heads develop into bladders, capable of forming brood-capsules containing scoleces—a fact as important practically as it is theoretically.[1]

The Cysticercoid stages of Bothriocephalus, known as Pleuracoids, are found in certain fresh-water fish. In Germany, Russia, Italy, &c., these parasites are harboured by varieties of pike, burbot, and perch; in Switzerland by the Salmonidæ; and in Sweden by the Coregonidæ. The Pleuracoids are situated both on and in the viscera, as well as in the muscular structure. When the body-cavity is opened, they appear as white thread-like structures, generally somewhat doubled up, and

[1] For further information regarding Cysticercoids. see p. 126.

1 to 2 cm. in length. They lie in the sexual glands, the liver, the mesentery, and under the peritoneal coverings of the intestine. They will also be found in the muscular structure, if this is cut with a broad knife tangentially into thin slices, and these slices are held either up to the light or over a dark background. The Pleuracoids are generally enclosed in thin-walled cysts, though "wandering" individuals are sometimes met with. The head is furnished with two suckers and is always invaginated. It will be projected, however, and the parasites will move actively about, if they are put into normal saline solution at mammalian body temperature.[1]

A certain number of the Cestodes of the domestic mammals approximate in their development to that of *T. solium*, L., the archetype of the genus *Tænia*. These are *T. crassicollis*, Rud., of cats; *T. serrata*, Gze.; *T. marginata*, Batsch; *T. cœnurus*, Küchenm.; and *T. echinococcus*, v. Sieb., of dogs. To these may be added *T. serialis*, Baill., which occurs in dogs in France, and probably also in Russia, though not in Germany; *T. krabbei*, Moniez, which is parasitic, probably in dogs, in northern latitudes; and certain varieties observed in wild mammals. The group is characterized by a homogeneous organization, and the specific distinctions are: the number, shape, and size of the rostellar hooks; the position, in ripe segments, of the uterus; and certain minor features not to be described here. All the members of the group pass through a bladder-worm stage of development, which is termed variously: "Cysticercus" of *T. crassicollis, serrata, marginata,* and *krabbei;* "Cœnurus" of *T. cœnurus* and

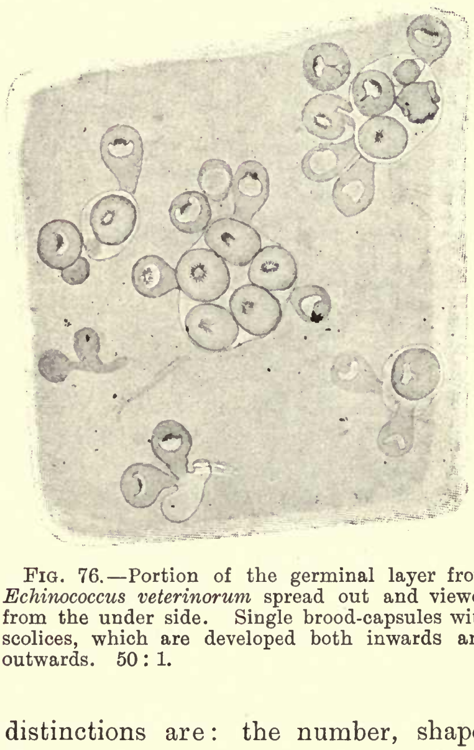

FIG. 76.—Portion of the germinal layer from *Echinococcus veterinorum* spread out and viewed from the under side. Single brood-capsules with scolices, which are developed both inwards and outwards. 50 : 1.

[1] When handling fish infected with Pleuracoids, or the flesh of cattle and swine infected with Cysticerci, great caution should be observed. Self-infection may result from the contact of the fingers with stray Cysticerci or Pleuracoids.

serialis; and "Echinococcus" of *T. echinococcus.* The Cœnurus forms many scoleces, while the Echinococcus forms brood-capsules and daughter-bladders, and from these many scoleces are formed.[1]

Herbiverous mammals harbour representatives of another group of Tæniidæ, the Anoplocephales. The most interesting are those of the horse, which are characterized by very short overlapping segments. They are *Anoplocephala plicata* (Zed.), *A. perfoliata* (Gze.), and *A. mamillana* (Mehl.). The species are distinguished by peculiarities in the structure of the head, which is invariably hookless. In *A. mamillana*, a worm of about 5 cm. in length, the head is small and is furnished with lateral elliptical suckers. In the other two species, on the contrary, the head is large and the suckers are placed upon its summit. The variety most commonly met with is *A. perfoliata.*

FIG. 77.—*Anoplocephala perfoliata* (Gze.). Natural size. (After Railliet.)

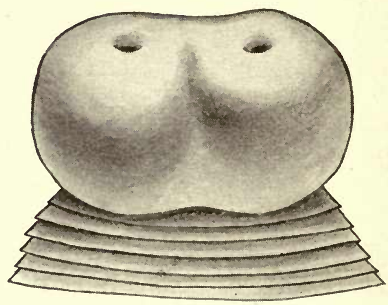

FIG. 78.—Head of *Anoplocephala plicata* (Zed.). 10 : 1. (After Railliet.)

This worm attains a length of 8 cm., and is characterized by four lobes, directed backwards, upon the head. In *A. plicata,* which grows as long as 80 cm., these appendages are absent. Owing to the length of the dorso-ventral diameter, the proglottides are difficult of examination. It is certain, however, that the formation of the genital organs begins in the segments next to the head, and is soon completed; that the genital openings are all situated upon the same margin; and that the genitals are arranged transversely of the pro-

[1] The bladder-worm of *T. crassicollis* (*Cyst. fasciolaris*) is found in the liver of rats and mice ; that of *T. serrata* (*Cyst. pisiformis*) in the liver of hares ; that of *T. marginata* (*Cyst. tenuicollis*) in the omentum of sheep; that of *T. krabbei* in the muscular structure of *Tarandus rangifer ;* and *Cœnurus cerebralis*, the Cysticercoid stage of *T. cœnurus*, is found in the brain of sheep, where it gives rise to the disease known as "gid." *Cœnurus serialis*, the bladder-worm of *T. serialis*, is found in hares and rabbits. The occurrence of Echinococcus has already been given above.

glottide, the testes being placed dorsally, and the ovary and yolk-gland ventrally, while between them lies the uterus, completely developed in ripe segments only. The eggs are large and bullate, or polyhedral as the result of mutual pressure, and the embryonal covering or pyriform apparatus is prolonged into two branching horns.[1]

The life-history of the Anoplocephales of the horse is entirely unknown, and this applies also to those of ruminants, of which a large number are known to us. In spite of the researches of Blanchard, Moniez, Perroncito, Railliet, Stiles and Hassall, and Cholodkowsky, our knowledge of the subject is still very limited indeed. The species of Anoplocephalines which occur in ruminants in Germany are: *Moniezia expansa* (Rud.), *M. denticulata* (Rud.), and a variety which was at first called *Tænia ovilla*, Riv., but was afterwards renamed *T. giardi*, Mon., and is provisionally included in the genus *Thysanosoma*, Dies. The two Monieziæ (of which *M. denticulata* usually occurs in cattle, while *M. expansa* is more frequent in sheep) are, like all Anoplocephalines, devoid of both rostellum and hooks. Each of the short, broad proglottides is furnished with a genital pore at each marginal edge, which communicates with the cirrhus-pouch and vas deferens or vagina. There are also two uteri, two shell-glands, two ovaries, and two yolk-glands. The latter are situated near the lateral margins, internally of the excretory canals, while the numerous small testes (seen in mature segments only) are distributed among the female organs, and do not, as a rule, present a bilateral arrangement. The shape of the embryonal sac is also very similar; in both species it is drawn out into two long, unbranched horns, which, in *M. expansa*, are 4 to 5 metres in length, while in *M. denticulata* they attain a length of 40 cm. The species are distinguished from one another by the length of these horns, the rate at which the proglottides develop, and the occurrence of interproglottidal glands. The latter are single-celled glands, which form at the posterior margin of the segment, and frequently occur singly. In *M. expansa*, however, they take the form of sacs, arranged in linear series and communicating with the body-covering (fig. 79).

Tænia giardi, Mon., may grow to a length of 2 metres or more and is usually furnished with alternating genital openings, though in some segments these are duplicated. The greater number of segments possess a single genital apparatus, placed internally to the excretory canals, but in some segments this may also be duplicated. The

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[1] *Cf.* Z. Kahane, "Anat. v. Tænia perfoliata" (*Z. f. wiss. Zool.*, vol. xxxiv, 1880, pp. 174-254; F. Zschokke, "Rech. sur la struct. anat. et hist. d. Cestodes," Genève, 1888; A. Scheibel, "Der Bau der Tænia magna Abbild." (*T. plicata*, Zed.), in.-Diss. (Giessen), 1895.

position of the testes, however, is very characteristic; in each segment they occupy a position in the narrow marginal fields external to the excretory canals, leaving the wide middle field entirely free. The latter is occupied by the numerous bullate diverticula of the uterus, which is placed transversely. The eggs, six to ten of which lie in each diverticulum, soon lose their shells, leaving the oncospheres surrounded by a simple chitinous envelope without appendage.[1]

Another group of the Tæniidæ, members of which infest the domestic mammals, is the genus *Dipylidium,* R. Lkt., which is described above. Tæniæ having the genital pores upon the flat surface, with sac-shaped uterus and unarmed head, are occasionally

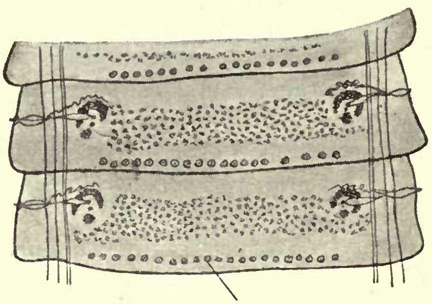

FIG. 79.—Two mature proglottides of *Moniezia expansa. Id.*, Interproglottidal glands; above these are the testes; at the sides, the female sexual glands may be seen.

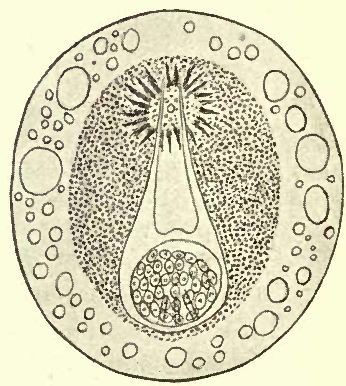

FIG. 80.—Egg of *Moniezia expansa,* very much magnified. (After Moniez.) In the centre, the oncospheres, surrounded by the pyriform apparatus, are seen.

met with in the cat and dog, though they are more frequent in the fox. These parasites belong to the genus *Mesocestoides,* Vaill. (= Ptychophysa Hamann).[2] *Dibothriocephalus latus* is found in dogs in localities only where this worm is prevalent, and it is encountered even less frequently in the cat. Of still rarer occurrence in the latter host is *Bothriocephalus felis,* a much smaller species of parasite. According to Krabbe, dogs in Iceland harbour Bothriocephalinidæ of species which are unknown to us.

The numerous Cestodes harboured by our domestic poultry belong,

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[1] C. W. Stiles and A. Hassall, " A Revis. of the Adult Cestodes of Cattle, Sheep, and Allied Animals" (United States Department of Agriculture, Bureau of Animal Industry, Bull. No. 7, Washington, 1893).

[2] F. Zschokke, "Zool. Anz.", vol. viii, 1885, p. 380, u.l.c., Genève, 1888; O. Hamann, *Z. f. wiss. Zool.*, vol. xlii, 1885, p. 718.

as a rule, to the genera *Hymenolepis* and *Davainea.* The *Hymenolepis* varieties are characterized: by the position of the genital pore, which is always at the left border of the segment; by the triple formation of the testes; by the presence in the vas deferens of an outer and inner vesicula seminalis; by the sac-shaped uterus; and by the single row of hooks upon the rostellum. The *Davaineæ* possess a double row of hooks upon the rostellum, together with several rings of hooklets leading into the suckers; the uterus, moreover, disappears, leaving one or more eggs in so-called parenchyma-capsules. *H. lanceolata* (Bloch) of ducks and geese is an excellent example of the genus *Hymenolepis.*

(3) Nematodes (Threadworms).

Living specimens of the larger Nematodes, such as *Ascaris megalocephala* of horses, *A. suum* of swine, and *A. lumbricoides* of man, are readily obtainable. They should be examined in the first instance with the naked eye.

The distinctive sexual characteristics will be apparent if adult specimens of both sexes are examined side by side. In both, the body is long and spindle-shaped and pointed at the posterior end. The male, however, is smaller and slighter than the female, and has the posterior end of the body recurved towards the ventral surface. The male differs also in having no special genital opening; the genital organs, which are termed "spicules" and which appear as two small, yellowish-brown rods, issue from the anus, which is placed close to the hinder end of the ventral surface of the body (fig. 81). In the female, the genital opening is also upon the ventral surface, situated at the border-line of the anterior and middle portions of the body and some considerable distance, therefore, from the anus. It will be seen with the naked eye if the body-surface, which is here slightly indented, is dabbed with filter-paper. The papillæ which surround the anal opening of the male will be seen only with a strong glass. The mouth-opening is terminal in both sexes and is surrounded by three papillæ or lips, one placed dorsally, two ventrally. Immediately behind the mouth papillæ, in the median line of the ventral surface,

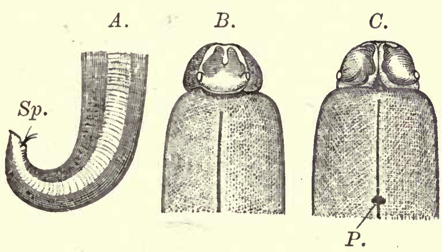

FIG. 81.—*Ascaris lumbricoides*, L. Male. *A.*, Posterior extremity. *B.*, Anterior extremity, dorsal aspect. *C.*, Anterior extremity, ventral aspect. *P.*, Excretory opening. *Sp.*, Spicula. (After Claus.)

is the small opening of the excretory system, generally only to be seen with the glass. In fresh worms, the coils of the genital glands are more or less distinctly seen; they appear as white threads through the thin body-wall. Running down each side is a broad light band (lateral line), while a narrow stripe (median line) runs down the dorsal and ventral surfaces.

The worm should be laid upon its dorsal aspect in a shallow vessel, and should be kept in this position by means of pins crossed above the body at the head and tail ends. It is opened with a pair of fine scissors in the median line along its entire length; the incision should be purely superficial, care being taken to cut nothing except the body-wall. If the incision is too deep, the intestine and excretory organs may be damaged. The incision should be continued round one side of the anus, and, in the female, round the sexual opening; the body-covering should be turned back and secured with pins, and the specimen will now be ready for examination. The process of preparation is exceedingly simple and may be successfully carried out by the beginner.

When seen from the inner surface, the body-wall appears to be covered with minute bumps. These are the protoplasmic parts of the longitudinal muscle-fibres of the body-wall. The body-wall is divided into four longitudinal fields with well-marked boundaries. Two of these boundaries are seen as longitudinal thickenings upon the inner surfaces of the body-tube, while, of the other two, the ventral boundary coincides with the line of incision, and the dorsal boundary is covered by the intestine and is only to be seen after this is removed.

Within the body-cavity lie the excretory and reproductive organs. The alimentary canal extends in a straight line from the mouth to the anus. It consists of the short oral cavity, surrounded by the papillæ; the whitish œsophagus, about 1 cm. in length and of an elongated bottle-shape; the long yellowish-green intestine, somewhat flattened dorso-ventrally; and the short rectum.

The sexes differ from one another, not only in the nature of the genital opening, but in the arrangement of the sexual organs. In the male, they are single; while in the female they are, with the exception of the vagina, invariably double. The male organs consist of a single thread-like testis and a ductus ejaculatorius; the female possesses two ovaries, two uteri, and a single vagina communicating directly with the genital opening. The student should uncoil these organs and compare their appearance in the two sexes. The male organs also include two small sacs, situated dorsally of the rectum and communicating with it, from the epithelium of each of which a spicule is secreted. The ova should be removed from the anterior ends of the uteri of sexually mature females, and should be examined separately under the microscope.

The macroscopic inspection of the living parasite should be supplemented by the preparation and examination of transverse sections. These should be cut from well-hardened Ascarides, either by hand or, after embedding in paraffin or celloidin, with the microtome. It is best to examine, first, a section taken through the anterior portion of the body, in front of the sexual organs and behind the œsophagus. Within the smooth cuticle is the subcuticular layer, containing cell-nuclei and fibres; it is heaped up into four thickenings, placed in the median and lateral lines. Between these thickenings, and divided by them into four fields, lies the muscular structure, composed of cells arranged longitudinally; these are severed transversely in the section. The muscle-cells are comparatively long. Each consists of a peripheral groove-like portion containing the muscle-fibrillæ, and of a protoplasmic portion continuous with the groove, which projects considerably into the body-cavity and contains the large nucleus. With this protoplasmic portion of the muscle-cells, the nerves are associated; they are seen in the section streaming out to right and left from the median cords. Longitudinal nerve-cords, of a slighter build than the median nerves, are placed at the sides, and close to them the lumens of the excretory canals will be seen. These canals unite at the level of the excretory pore, close behind the mouth papillæ, to form a single channel. In the centre of the section and distinguished by the columnar structure of its epithelium, is the intestine.

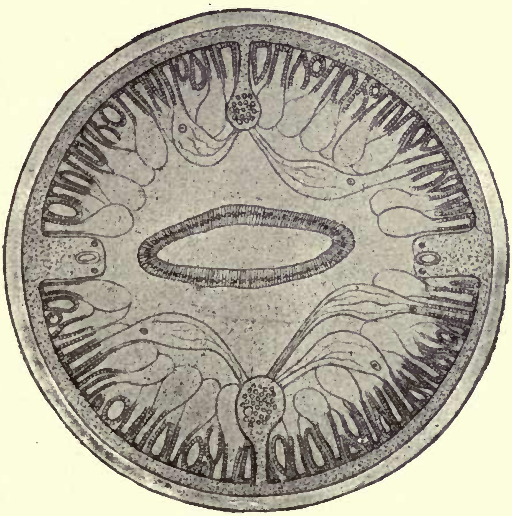

FIG. 82.—Diagrammatic representation of a transverse section through *Ascaris lumbricoides.* In the centre is the intestine, flattened dorso-ventrally; to right and left, in the body-wall, are the lateral lines, enclosing the excretory vessels and the lateral nerves; at the top and bottom of the central plane are the dorsal and ventral median lines, showing the nerve-fibres and the manner in which they branch out towards the muscular structure. 50 : 1. (After Brandes.)

A second section should be taken from the anterior portion of the body at the level of the œsophagus. The œsophagus is seen to be a thick-walled tube with triangular lumen, which is lined with a continuation of the cuticle; the walls consist of muscle-fibres arranged

for the most part radially. If the section is taken in front of the genital pore, the circumœsophageal nerve-ring will be seen, and will appear as if hung upon the four longitudinal lines.

The mouth papillæ should be cut off and prepared for the microscope. When seen from above, they are nearly heart-shaped, and where the surfaces approach one another they are furnished with a very fine dentition and with sense-organs. These appear as lighter patches, two of which are placed upon the dorsal, and one each upon the ventral, papillæ.

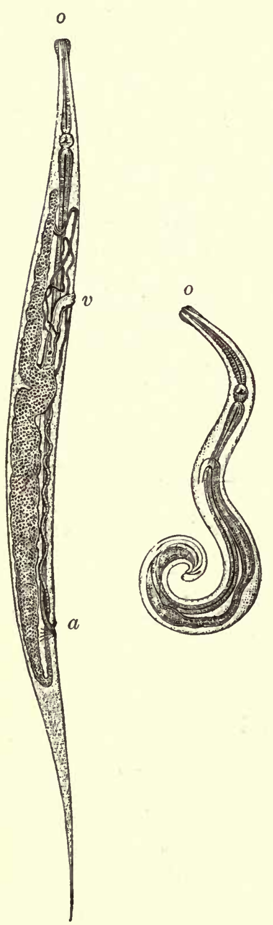

FIG. 83.—*Oxyuris vermicularis.* Left, female; right, male. *a*, Anus. *o*, Mouth. *v*, Vulva. Magnified. (After Claus.)

Cross-sections from the male at the level of the sexual glands show, between the intestine and the body-cell, numerous transverse and diagonal sections of the coiled, thread-like testes. Similar sections from the female will show the ovary, likewise cut across in various directions; the two uteri, divided transversely; and the lumens of the oviducts, which lead from the ovaries to the uteri. Sections of the female organs are subject, however, to considerable variation.

A section should be taken from the posterior end of a male Ascaride, a little in front of the anus. In the centre is the intestine; above it, the two spiculum sacs are placed symmetrically; ventrally from the intestine is the ductus ejaculatorius.

The Ascarides are not, however, the only Nematodes which are deserving of attention. There are numerous varieties belonging to other genera, both parasitic and free-living, which, on account of their transparency and small size, are eminently suited to microscopic investigation, and these are readily obtainable. The parasitic forms occur in the intestine of fish, frogs, birds and mammals; while the free-living forms are found in mud, or between decaying leaves in water. Those varieties which live in damp earth may be attracted by pouring milk or blood on the ground, or, after removing a patch of turf, by burying a piece of meat just below the surface. The Nematodes will appear in quite a short time, sometimes after a few hours only, and *Rhabditis pellio* is almost certain to be

among their number. This species, with others related to it, possesses the property of facultative parasitism, and has, on rare occasions, adopted man as its host.

Of the parasitic Nematodes which occur in Central Europe, the one most readily obtainable is *Oxyuris vermicularis.* It is frequently harboured by children, especially in the country, and is voided in large numbers with the fæces. The well-known "threadworms," which move actively about upon the surface of the warm fæces, are female Oxyures, the uteri of which are full of eggs containing embryos. They should be handled with the greatest caution, as at this stage they are very infectious.

Trichinous pork is also an infective material which is easily procured, though it is by no means as common now as formerly. It should be cut parallel with the muscle-fibres into small pieces, and these should be pressed between two glass slides. The Trichinæ are spirally coiled worms enclosed in lemon-shaped capsules ; they are easily discoverable with a low power lens. Infection is rarely so extreme that parasites are present in every piece of flesh, and it is generally necessary to examine a large number of specimens before finding proofs of disease. Even where infection is very advanced, large portions of the muscular structure are frequently free from parasites, though these may be present in large numbers in other muscles of the host (diaphragm, pharynx, lingual, and intercostal muscles), or in other parts of the same muscle, as, for instance, the point of attachment of the tendons. Trichinæ are very difficult to discover in flesh which has been converted into sausage-meat, and detection here is entirely a matter of chance; hence, a negative finding is no proof of immunity.

In full-grown swine, the encapsuled Trichinæ begin to calcify about six to nine months after infection, though the process may begin earlier, and it is, occasionally, omitted altogether. It commences at the poles and about fifteen to sixteen months are required for complete calcification of the capsule, which leaves the inmate unaffected. This process of calcification, which extends in the course of years to the inmate of the cyst, is part of the normal development of the parasite and is rarely observed in swine, as these are generally slaughtered in their first year. There is an analogous pathological condition in which the worm dies and calcifies, either before or after the formation of the capsule, the process of calcification subsequently spreading to its surroundings. It should be borne in mind that the Cysticerci which occur in the flesh of swine may also die and calcify. They are readily distinguishable from calcified Trichinæ by their larger size and different shape, as well as by the presence of the cyst, which is composed of connective tissue of varying thickness.

If rats or mice are fed with trichinous flesh, sexually mature Trichinæ and their progeny will be found in the intestine after the interval of a few days. The wanderings of the parasites, their penetration into the muscular structure, the changes to which they give rise, and their subsequent encystment, all furnish interesting material for investigation.

Of the other Nematodes which, in Central Europe, are parasitic in the intestine of man, the most important are: *Trichocephalus trichiurus*, which inhabits the cæcum and is frequently met with in certain localities, and *Ankylostoma duodenale*, formerly very prevalent among miners, but now less frequent. Both of these forms (which belong to different families, the first being a Trichotrachelide, the second a Strongylide) are either of sufficient transparency in the fresh state to permit of examination whole, or they may be rendered so by suitable treatment. Nematodes very rarely occur in the lungs of man, though they are found with considerable frequency in the lungs of the domestic animals and of game. Filaria are equally rare in man in these latitudes, but *Filaria equina*, found in the body-cavity of the horse, may be used for experimental purposes. Blood filariæ should be sought in the blood of crows (*Corvus* varieties).

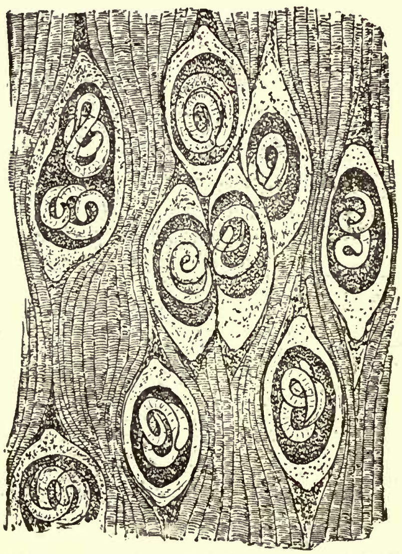

FIG. 84.—A piece of trichinous pork, showing a very high degree of infection. Magnified.

The Nematodes of the domestic mammals should be examined by the method described. The anatomy of the larger species (*Ascaris megalocephala*, Cloq., of horses, *A. vitulorum*, Gze., of calves, *A. suum*, Gze., of swine) is best studied by means of sections taken from worms which have been well hardened. In the case of the smaller varieties the whole worm, either fresh or after clearing with glycerine, will usually furnish sufficient data. The number of species known to us has become so large that it is not possible to name them, far less describe them, here. It will suffice to give the names of the families to which they belong. To the Ascaridæ belongs the genus *Oxyuris*, examples of which are *Oxyuris curvula*, Rud., and *O. mastigodes*, N., found in the large intestine of the horse. Of the Strongylidæ, some inhabit the

intestine, as *Strongylus contortus*, Rud., in the abomasum of sheep and cattle; *S. ostertagi*, Stiles, under the epithelium of the abomasum of cattle; *Sclerostomum armatum*, (Rud.), and allied species, sexually mature in the intestine of horses, as larvæ in the arteries of the abdomen, where they give rise to verminous aneurism; *Ankylostoma trigonocephalum* in dogs; and other varieties. Other Strongylidæ inhabit the lungs, as: *S. rufescens*, Lkt.; *S. filaria*, Rud., in sheep; *S. apri*, Gm., in swine; and other varieties. With the exception of *Trichinella*, the Trichotrachelidæ are represented by: *Trichocephalus crenatus*, Rud., in swine; *T. affinis*, Rud., in the sheep, goat and ox; *T. depressiusculus*, Rud., in the dog. Examples of the Filariæ are provided by *Filaria equina*, Abild., in the body-cavity of the horse; *F. immitis*, Leidy, in the heart of the dog; and other varieties. *Gnathostoma hispidum*, Fedtsch, is found in the stomach of swine of Hungarian origin; the male of this species is up to 25 mm. long, while the female may attain a length of 31 mm.

(4) Acanthocephala (Hooked Worms).

The Acanthocephala are found in the intestine of vertebrates. The smaller species have considerable transparency and are well suited to a first examination, the details of their structure being clearly visible with a low power lens if they are pressed between two glass slides. Material will be found in the intestine of frogs and of fresh and salt-water fish. The parasites are furnished with an armed cylindrical proboscis, by means of which they attach themselves to the bowel-wall; they are, however, easily detached. The trunk is of a white or reddish-yellow colour, and, in the living specimen, is usually quite flat with irregular transverse wrinkles. When put into water or other liquid it swells up and becomes cylindrical, but does not lose its transparency. As soon as the worm is removed from the host, it should be put on to a cover-glass with a little normal saline and examined. If the proboscis is withdrawn, the worm should be squeezed with the fingers, the pressure being directed from the thin posterior end forwards, and this manœuvre should be repeated until the proboscis appears. A cover-glass should then immediately be placed upon the worm, and if this is insufficient to prevent the reinvagination of the proboscis, the pressure should be increased.

The Acanthocephala are, without exception, diœcious. The sexes are distinguished in the first place by difference in size, the male being considerably shorter than the female. The anterior end of both is somewhat thickened and is crowned with a rostellum, which may be cylindrical, ball, or club-shaped, according to species, but is invariably furnished with rings of hooks. The number, shape and

size of these hooks have a certain specific value. In many varieties a narrow neck-piece is interposed between the proboscis and the trunk, and the latter frequently bears a varying number of bristles.

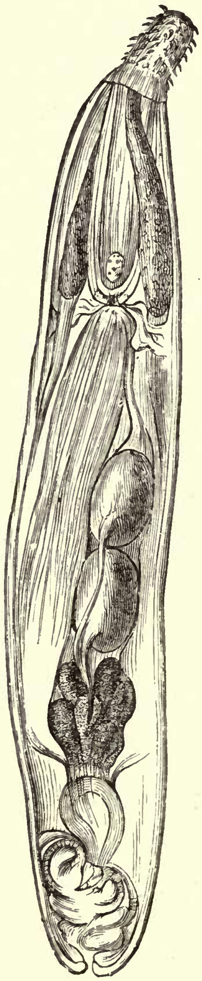

FIG. 85. — *Echinorhynchus augustatus.* Male, 25 : 1. Of the three organs placed immediately behind the proboscis, the central one is the proboscis sheath, those on either side the lemnisci. The retractor of the proboscis sheath takes its rise at the base of the sheath. By it, to the right, are the oval testes; behind them, the darker cement-glands; and behind these again, the penis. (After Leuckart.)

The body-wall is comparatively thick. Through it, either in patches or extending over the entire body, a network of lighter channels can be seen. This network generally proceeds from two main stems, and has no apparent communication with the exterior. It runs from the proboscis, through the neck into the trunk, where it is continued into two appendages of the body-wall, known as the lemnisci. These are saclike structures, dissimilar in size and shape, which project into the body-cavity. A fluid circulates in the canal system, derived from the contents of the intestine; it contains a varying proportion of granules.

Within the anterior portion of the body-cavity, situated in the axial line, is a long sac-shaped organ with thick muscular walls; this is the proboscis sheath. Its function is twofold; it acts as a receptacle for the proboscis when withdrawn, and the extrusion and evagination of the proboscis is effected by means of the muscular contractions of its walls. The proboscis is retracted by means of a longitudinal muscle running in the axis of the sheath, one end of which is attached to the body-wall, while the other end is attached to the inner surface of the distal end of the proboscis. A second longitudinal muscle is attached to the base of the sheath, and runs diagonally across the body-cavity in a backward direction, to a point of attachment in the body-wall (retractor receptaculi) (fig. 85).

In those species in which the walls of the proboscis sheath are thin, a rounded mass of cells will be made out near its posterior extremity. This is the nerve ganglion and from it the nerve-cords, usually

three in either direction, run forwards into the proboscis and backwards into the trunk.

The only other parts of the internal structure which are visible in the living specimen are the sexual organs. Very little of these can be seen, however, in sexually mature females, the body-cavity being more or less completely filled with germinal cells, cell masses, and eggs enclosed in shells. In the middle of the body of the male the two oval testes will be seen, placed one behind the other. Their ducts, which are directed backwards, are seen to combine and are surrounded by the cement glands, club-shaped bodies, usually of a very dark colour. Beyond the cement glands the vas deferens passes into the muscular penis, which is withdrawn into the genital bursa. The bursa is a bell-shaped organ situated at the posterior end of the body, with an orifice communicating with the exterior. It is occasionally seen to be everted and in this position it plays a definite part in the act of copulation. The genital opening of the female is also situated at the posterior end of the body.

In the young female, the ovaries are in a position analogous to that of the testes in the male, but they soon break down into a number of cell agglomerations, known as "loose" or "floating" ovaries, and sometimes termed "placentulæ." At the period of sexual maturity, successive single cells (egg-cells) are released and, after fertilization, these develop within the body-cavity into the eggs. The latter are usually spindle-shaped; they are enclosed in three shells, and they contain a finished embryo. They more or less completely fill the body-cavity, concealing the apparatus by which they are ejected, the orifice of which is situated at the hinder end of the body. This apparatus should be studied in young females, or a mature female may be opened longitudinally and all loose structures removed by means of a water-jet, when a long, cordlike structure will be seen. This should be removed and examined separately, when it will appear as a tube, open at both ends, and divided into three parts of unequal length. The central portion, the uterus, is the longest; it is prolonged backwards into the short vagina which communicates with the exterior, while its anterior end is continuous with the bell, an organ whose shape varies in different species. The bell is provided with three openings: one placed anteriorly and opening into the body-cavity; one placed posteriorly and opening into the uterus; and a third, which is situated in the ventral wall. The bell-wall is muscular at its anterior end but becomes cellular before passing into the uterus. It performs certain swallowing movements which may be seen by examining the freshly removed organ, together with a portion of the body contents, in white of egg. By means of this movement the bell swallows a number of the bodies floating in the body-cavity and

passes them on into the uterus. The lumen of the hinder portion of the bell is, however, of such a size that it will admit only bodies of a certain shape, that, namely, of the enclosed ova. Bodies of other sizes and shapes are returned through the ventral opening into the body-cavity. It happens, however, that numerous enclosed ova are returned to the body-cavity, with the unsuitable bodies, to be again swallowed by the bell. This manœuvre is continued until the stream finally compels them to enter the hinder part of the bell, whence they are passed on into the uterus.

The entire sexual apparatus of the male will be seen to be enclosed in a sheath, known as the "ligament" (ligamentum suspensorium), which takes its rise at the base of the proboscis sheath. A similar ligament is present in the female, the posterior end of which enters the bell and is attached to its base. In young worms it encloses the ovaries, but when these break up and the placentulæ escape into the body-cavity, the ligament in the greater number of cases is ruptured.

Echinorhynchus gigas, the giant Acanthocephalide which inhabits the intestine of swine, is very useful for demonstration purposes. Its structural scheme differs from that of the smaller varieties in certain definite points, the more important of these being: the structure of the proboscis sheath and the bursa; the presence of two retractor muscles instead of one; and of eight cement glands instead of six. In the female, the ligament is composed of two tubes, in which the placentulæ remain and in which the eggs undergo development, not, as in other species, in the body-cavity. This arrangement presupposes a direct communication between the two ligament tubes (which also communicate upon their inner surfaces) and the oviducts, which is brought about in the following manner: the dorsal half of the ligament passes into the mouth of the bell, while the ventral portion is associated with the orifice from which the immature eggs are thrown out, and projects backwards and beyond the orifice in the form of a blind sac. The eggs of *E. gigas* diverge from the typical Acanthocephaline shape (fig. 43).

The ova of Acanthocephala are deposited within the body of the host and reach the exterior in the fæces. Each contains a finished embryo, of which, in spite of the transparency of the shell, scarcely more than the hooks situated at the anterior edge is visible. The shell may be burst open by means of pressure upon the cover-glass, but this is very likely to injure the embryo. An infinitely better method, and one first adopted by R. Leuckart, is the artificial infection of suitable intermediate hosts; the larvæ will then emerge spontaneously from the shell and will be found free in the intestine. In favourable conditions it is sometimes possible to catch the larva, when under the microscope, in the act of emerging.

Asellus aquaticus is the intermediate host of *Echinorhynchus angustatus*, which inhabits the carnivorous fish of these latitudes, and also of *E. hæruca* of frogs. *Gammarus pulex*, the water-flea, harbours the intermediate stage of various species, one being *E. proteus* of fish. The larvæ of the may-chafer (*Melolontha vulgaris*) and of the gold-chafer (*Cetonia aurata*) are the intermediate hosts of *E. gigas*. *Asellus* is an easy subject for experiment, as it may be obtained in large numbers and will live in aquaria.

The entire complicated developmental change occupies two or three months or even longer, according to temperature. It is not possible to describe here the process by which this change is accomplished, especially as certain of its details are still in dispute. The student is referred to the literature of the subject,[1] with the study of which he is strongly advised to combine the preparation and examination of sections in series.

Note : Hirudinea (Leeches).

Hirudo medicinalis may be procured from any apothecary; such animals are, however, usually empty, and when in this condition are not suitable subjects for experiment. Leeches should be kept in a glass bowl containing water, and a cloth should be tied over the top. Shortly before examination they should be fed on frogs, which should be introduced into the vessel with the leeches. The wound inflicted by the leech upon the body of the frog should first be examined; it will be seen to be three-rayed, the semi-circular edges curving inwards. The leeches should be killed in a wine-yellow solution of chromic acid, to which a few drops of glacial acetic acid has been added. They will contract very strongly, and as soon as they have ceased to move they should be stretched out with the fingers. They should be allowed to remain in the fixing fluid for a few minutes longer and then taken out, rinsed in water, and the external appearances should be studied.

The body is long and narrow with a plane ventral surface, towards which the terminal suckers are directed. The dorsal surface is somewhat more arched, and the two aspects differ in colour. The hinder end is recognized by the large disc-like sucker, while at the head end, which is somewhat narrower, is placed the small, slightly projecting anterior sucker. The opening of the intestine is situated upon the dorsal surface in the middle line, just above the large sucker. By opening the oral sucker with a pair of tweezers, three whitish

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[1] See Leuckart, Kaiser, Hamann.

swellings, placed radially, will be seen (fig. 87, *a*); these contain the jaws.

The body covering shows circular furrows placed very close together, which divide the surface into a number of narrow annulations. In *H. medicinalis* there are 102 annulations; but, with the exception of the anterior two and the one which bears the anus, they do not correspond to any segmentation of the body-cavity. In the larger portion of the body, consisting of 17 segments, 5 annulations go to make up a body segment, while the anterior segments are composed of 3, the posterior of 2 (or 1) annulations respectively. It requires very minute examination to make out the margins of the body segments from the exterior. Indications are offered by the sensory organs of the skin,[1] which are placed, 6 to 8 dorsally and 6 ventrally, upon the first annulation of each segment; and by the orifices of the 17 pairs of excretory organs (nephridia, segmental organs, looped ducts), placed at the sides of the ventral surface upon the last annulation of each segment, and only to be seen with the glass. The sexual openings are more easily found. They are placed upon the ventral surface in the median line; the male orifice, from which the white thread-like penis frequently depends, being situated in the 10th body segment, between the 30th and 31st annulations; while the female orifice is behind it, in the 11th segment, between the 35th and 36th annulations.

FIG. 86. — Showing the anatomical structure of Hirudinea. The dorsal surface has been removed and a portion of the intestine, between the penis and the first pair of testes, is cut away, showing the female sexual organs. The loops at the lateral margins represent the nephridia. (After Kennel.)

In all Hirudinea the width of the body-cavity is reduced by longitudinal canals, resembling blood-vessels in appearance, which run the entire length of the body and are not anatomically distinct from the body-wall. The presence of these canals renders the task of exposing the organs somewhat difficult. The leech should be opened upon the dorsal aspect, care being taken not to penetrate the intestine. A good deal of practice is required to perform this manipulation successfully.

The best method is to lay the parasite upon its ventral surface and, after stretching, to secure it by

[1] Upon the 1st, 2nd, 3rd, 5th, and 8th annulations, two of the dorsal organs are, in each case, replaced by eye-marks. When seen with the magnifying glass, these appear as black spots with a regularly defined margin.

means of pins through the suckers. An incision is made in the middle line along the entire length of the dorsal surface. This incision must be kept purely superficial, or the intestine, which is attached to the body-wall by means of a brownish tissue, will be severed. The intestinal wall is whitish in colour, the dark contents showing through it. If the wall is injured, the blood which the intestine contains will immediately well out and the operator is warned that he has penetrated too deeply. The cut edge of the body-wall is slightly lifted with the tweezers, and the connective tissue between it and the intestine is severed with a pair of fine scissors by means of small horizontal cuts. When freed, the intestine will be found to have eleven pairs of blind sacs attached to it. Of these pairs of sacs, ten are arranged diagonally backwards; while the eleventh pair, which is much longer than the others, run in a backward direction upon either side of the end-gut and terminate just in front of the anus. Between the saccular structures, and outside the hinder pair more particularly, portions of the nephridia will be seen; and if the walls are freed well towards the periphery, the two so-called lateral canals (lateral cavities) will be exposed. The structural difference between the anterior and posterior portions of the intestine will be apparent to the naked eye. In the anterior portion the walls are thicker (ring muscles) and numerous muscle-fibres, starting from the body-wall, are arranged radially round the spindle-shaped pharynx. The function of the pharynx is to suck blood from the wound inflicted by the jaws and to force it into the alimentary tract.[1]

As soon as the intestine is freed, it should be severed behind the pharynx and removed, care being taken not to injure the organs lying beneath it. At the sides of the body-cavity will be seen the seventeen pairs of nephridia with their terminal sacs; in the median line is the ventral nerve-cord, the anterior end of which lies hidden beneath the pharynx; between the nerve-cord and the lateral canals lie the testes, nine upon either side, furnished with short, laterally directed ducts attached to the vasa deferentia, which run from back to front; in front of the anterior testes lie the small ovaries and, between these, the two short oviducts are seen, leading to the thicker uterus, which communicates with the exterior by means of a short vagina. The two vasa deferentia are continued forwards over the female genitals; their walls become thicker and they coil themselves into the so-called

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[1] There is another method of preparing leeches which shows the alimentary canal with great clearness. The leech is killed in chromic acid, and weak chromic acid solution is introduced by means of a syringe through the pharynx into the canal. The worm should be stretched, hardened, and divided into dorsal and ventral halves by means of a longitudinal section.

epididymis ; they then form the muscular ductus ejaculatorius, which is continued on to form the penis. The base of the penis is swollen into a kind of bulb which contains numerous prostate glands.

With regard to the nervous system, it will be sufficient for the student to make himself acquainted with its anterior portion, that is to say, with the circumœsophageal ring. The pharynx should be carefully loosened without damaging the nerve-cord lying beneath it. The longitudinal section of the body-wall should be continued forwards until the pharynx is quite freed from the dorsal wall. If the pharynx is now pulled sharply backwards the two upper pharyngeal ganglia will be seen lying upon it. They will appear as two rounded blackish bodies of about the size of a grain of linseed. If the pharynx is now cut through above the two ganglia and pulled forwards, the entire œsophageal ring will be seen. The central nervous system is remarkable for the fact that the ladder-like arrangement is modified by the position of the ganglia. The two ganglia of each segment are so close together that they appear to be one. Owing to the reduction in length of the segments to which they belong (seen externally by the smaller number of annulations), the anterior and posterior ganglia are also very close together, while those in the fully developed five-ringed segments are considerably farther apart.

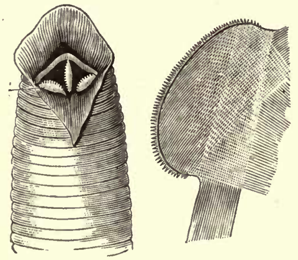

Fig. 87. — *a*, Anterior end of *Hirudo*. The oral cavity is open and shows the three jaws. *b*, A jaw with muscular attachment very strongly magnified. (After Claus.)

The three jaws should be removed from the oral cavity and examined under the microscope upon a glass slide. They are plane objects, nearly semicircular in shape, the curved edge being furnished with minute teeth, which may be seen with a low-power lens. The number of these teeth varies within certain limits. Between them are the orifices of the salivary glands, the secretion from which prevents the coagulation of blood.

Sections should be prepared from leeches which have been well stretched and thoroughly hardened. The animals should be killed in acetic acid solution of chromium, stretched, cut into several pieces, which are returned to the fixing fluid and allowed to remain in it for several hours ; these are then rinsed, dehydrated, hardened in alcohol by graduated stages, and cut, either by hand or with the microtome. A transverse section taken from the middle of the body will show the following conditions : externally is a thin cuticle, beneath it a single layer of epithelium, between the cells of which pigmented connective tissue and blood-vessels are sometimes seen ; within this is a thinner

layer of ring-shaped muscles; and within this again, a thick band of longitudinal muscles, which appear in the section as transversely cut tubes. The space between the muscular structure of the body and the intestinal wall is filled with connective tissue, containing many pigmented cell agglomerations. In the centre of the section is the intestine, more or less completely filled with blood, flanked by the intestinal diverticula, which are severed transversely. Upon the dorsal aspect of the intestine is the dorsal vessel; ventrally of it, the ventral cord; and between the intestinal diverticula and the periphery are the lateral vessels. The ventral field also contains the excretory vessels in transverse or diagonal section, as well as sections of the testes, placed right and left of the ventral cord.

Sections taken at the level of the pharynx or of the genital orifices illustrate very different conditions. Median sections through the anterior end are particularly interesting, as they show the structure and the method of function of the organs employed in the sucking act.

PART III.

ARTHROPODA.

To the student of microscopic parasitology, the interest attaching to the parasitic Arthropods centres chiefly in their external appearances, as these have a diagnostic significance. For this reason the descriptions here given are confined to details of the external body-form and structure, though it is obvious that they may incidentally reflect conditions governing the internal organization.

The body of the Arthropod, together with its appendages, is covered with a cuticle of varying thickness, which consists of a chitinous substance of extreme resistance. Where the animal is opaque, or nearly so, it is necessary to isolate what is known as the "chitinous skeleton."[1] This is done by maceration in a weak (10 per cent. or less) solution of potassium, and the action of the fluid may be hastened by warming or, better still, boiling. The object should first be killed in alcohol and then transferred by graduated alcohol and water stages to pure water.[2] The maceration of the more fragile varieties requires careful management, as boiling, especially if continued for any length of time, will not only cause the slender body appendages to drop off, but may give rise to pronounced structural changes and so render the specimen useless. The best method of treating delicate objects is to allow them to macerate in potassium solution at room temperature; large varieties will take several days, or even a week or more, smaller specimens proportionally less. If,

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[1] According to G. Enderlein (*Zoolog. Anz.*, vol. xxvii, 1904, p. 497), small dried objects may be prepared for examination in the following manner: one part of a "moderately strong" potassium solution is mixed with eight to ten parts water, and the objects are allowed to soak in this mixture until they have approximately regained their normal shape. This will take from ten minutes to several hours, according to the size and delicacy of the objects. They should be rinsed in water and the larger air-bubbles smoothed out with a fine camel's hair brush. They are returned to the solution, again rinsed in water, and dehydrated by means of the alcohol stages. They should be finished for the microscope by the methods described above.

[2] When dealing with the larger varieties, the action of the drug should be hastened by pricking the specimens with a fine needle. The site selected should be without special structural significance, as, for instance, a segmental margin in forms with segmented abdomen.

for any reason, it is desired to hasten the process, the specimens should be placed in a thermostat at a temperature of 40° or 50° and allowed to remain there for half a day or one day. For boiling, a water-bath should be used, into which the glasses containing specimens and solution are plunged. In the case of the larger varieties the process will take several hours.

As a general rule, the objects are sufficiently macerated when they begin to acquire transparency. The remains of the solution, together with the softer body-parts, which will now have become soluble in water, are rinsed out with distilled water, to which a drop of glacial acetic acid has been added. The solution is poured off and replaced by the acidulated water, which is changed repeatedly until the objects become light and clear, the process being watched under the microscope. The objects are now taken out of the water and laid upon a glass slide, some with the dorsal, others with the ventral, surface uppermost. If there is only one specimen, it should be first viewed from the ventral aspect. The object should be arranged, with the aid of the microscope or strong magnifying glass, with a fine brush; the extremities should be extended, and the water removed with filter paper. Props of suitable thickness should be conveniently placed and the specimen covered with a cover-glass. Weak alcohol should now be very carefully added and replaced, after a short interval, by stronger alcohol. If the objects are to be mounted in balsam, the cover-glass should be removed as soon as they have become hard; they should be dehydrated with strong alcohol, cleared with oil of cloves, and then mounted. Delicate objects become too clear when mounted in balsam, and these should be put through the alcohol and glycerine stages to pure glycerine; or the chitinous substance may be coloured with earmine or aniline stains; or Mayer's mixture (a solution of pyrogallic acid in alcohol or glycerine) may be used. Superfluous colour is removed with a weak acid solution, and the specimens should be mounted in balsam, glycerine, or glycerine-gelatine.

Maceration in potassium solution may be omitted in the case of small, transparent, slightly coloured parasites, but only when these are obtained alive. They should be put on to a glass slide, covered with a cover-glass, and, as soon as their legs are extended, killed with alcohol,[1] which should be introduced under the edge of the cover-glass. They are then finished and mounted in either glycerine or balsam. On account of the impermeability of the chitinous envelope, specimens which are to be mounted in balsam must be carefully

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[1] The legs of the larger varieties of Acarina will usually become extended if the parasites are killed in boiling water. For the smaller varieties a mixture consisting of 2·5 glycerine, 4·0 glacial acetic acid, and 93·5 alcohol, should be employed.

dehydrated with alcohol in stages of gradually increasing strength. Otherwise, water will be retained in the tissues, and the objects, when mounted, will be more or less opaque and, in this condition, useless. Oil of cedar-wood is frequently used as a clearing reagent, but in this case also the specimens must be very carefully dehydrated, as the oil absorbs water very readily.

(1) Acarina (Mites).

Of the Acarina which are known to be parasitic in man, two only, *Sarcoptes* and *Demodex*, are of permanent parasitic habit. All other varieties are occasional in their occurrence. They may be either the permanent or the occasional parasites of animals, adopting man as an accidental host; or they may be free forms, living in and on organic substances and attacking man only under certain favourable conditions. The greater number of species, including *Sarcoptes* and *Demodex*, are so extremely small that their examination is a matter of some difficulty to the beginner. For this reason it is advisable to begin with the group Ixodidæ, certain members of which attain a comparatively large size. Two species of Ixodidæ are readily obtainable in almost all parts of Central Europe; they are *Ixodes ricinus* (L.) and *I. hexagonus*, Leach, commonly known as wood-lice, or dog-ticks. They live in the leafy undergrowth of woods and plantations where, in order to obtain nourishment, they seek to attach themselves to one of the higher vertebrates. Thus, their most frequent hosts are wild animals (game), grazing cattle, dogs, and man.[1]

Specimens of Ixodides do not by any means all look alike. The empty tick changes considerably in appearance when the intestine becomes filled with blood and is in process of digestion. Moreover, there is considerable difference between the larva, the nymph, and

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[1] In addition to the above-named, the following varieties are met with in Germany: *Ixodes tenuirostris*, G. Neum., on *Arvicola* varieties in England and the Isle of Rügen; *Hæmophysalis punctata*, Can. et Fanz., on grazing cattle and game animals, in districts bordering the Mediterranean, in Holland and in the neighbourhood of Nuremberg; *H. concinna*, Koch, on grazing cattle and game, in France, Austria and Brunswick; *Dermacentor reticulatus* (Fabr.), on grazing cattle and game, in South and West Europe, in Asia, in North and Central America and in Würtemberg; and *Argas reflexus* (Fabr.), the European hedge-tick or pigeon-tick, found in pigeon-cots and on pigeons, rare in Germany. Tho other varieties which are said to occur in Germany are probably developmental stages of either *Ixodes ricinus* or *I. hexagonus*. The Ixodidæ have attracted considerable attention within the last few years; since, in fact, it has been recognized that, like *Anopheles*, *Culex* and *Glossina*, they are the carriers of infectious diseases (Texas fever of cattle).

the adult male and female tick. The larvæ are provided with six legs and are without sexual opening or stigmata. The nymphs have eight legs and are provided with stigmata, but are without sexual opening. The sexually mature male and female ticks have eight legs, and are provided with both stigmata and sexual openings. The number of eggs deposited by the adult female is very large. As soon as the larvæ emerge they attach themselves to lizards, very small mammals of widely different species, and birds. They suck the blood of their hosts but do not appreciably increase in size. When they return to the ground they slough their outer skin and enter upon the nymph stage. The nymphs, like the larvæ, remain for some time attached to small mammals, and upon leaving their host they enter upon the stage of sexual maturity. The male and female become parasitic upon the larger vertebræ, where copulation takes place. The body of the female becomes very much distended, partly from the filling of the intestine with blood, and partly as a result of the development of numerous eggs (fig. 88). She finally quits the host in order to deposit her eggs upon the ground. Females which are very much distended and are upon the point of laying eggs are not good subjects for demonstration purposes.

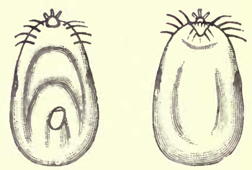

FIG. 88. — *Ixodes ricinus* (L.). Female, gorged with blood. Seen right, from the dorsal, left, from the ventral surface, 2 : 1. (After Pagenstecher).

Specimens are killed in alcohol, and this must be done under the cover-glass, or the extremities will not be extended. They should be viewed first with a magnifying glass or with a low power microscope. The body is unsegmented and there is no external line of demarcation between the cephalo-thorax and the abdomen. The legs are attached to the ventral surface. In males and young females, the body is oval; in older females it is nearly oblong; while in quite old females the oval outline is resumed. The dorsal surface is arched, the ventral surface plane, and it is customary to distinguish an anterior, a posterior, and two lateral marginal edges. At the anterior end of the body, immediately in front of the first pair of legs, is the capitulum, or "false head," which bears the mouth-parts. These consist of a proboscis-like structure, flanked on either side by the palps. Upon the dorsal surface, immediately behind the capitulum, is the arched scutum or shield, in colour of a varying shade of brown. With the exception of a narrow margin at the hinder end and at the sides, the dorsal surface in the male is entirely covered by the scutum. In the female, the scutum is much smaller and is of a different shape. The difference in size is noticeable even in quite young females, and

is very pronounced indeed in older, distended females, where the proportion of the dorsal surface covered by the shield is very small. The ventral surface presents other points of difference, being almost completely covered, in the male, with scales, which are separated from one another by lighter stripes. In both sexes the ventral surface bears two openings, placed in the middle line. In front is the transverse genital opening, and behind it, near the posterior margin, is the anus. Upon each lateral margin, just behind the legs, is a rounded stigma (peretreme), which appears to be porous, and is surrounded by a thickened edge. In the centre of each stigma is an opening, the spiraculum, which communicates with the tracheæ. The stigmata of the male are oval, those of the female circular. The scales upon the ventral surface of the male have a certain specific significance. Those in the median line are the pregenital, genito-anal, and anal scales; while the adanal and femoral scales are duplicated upon either side.[1]

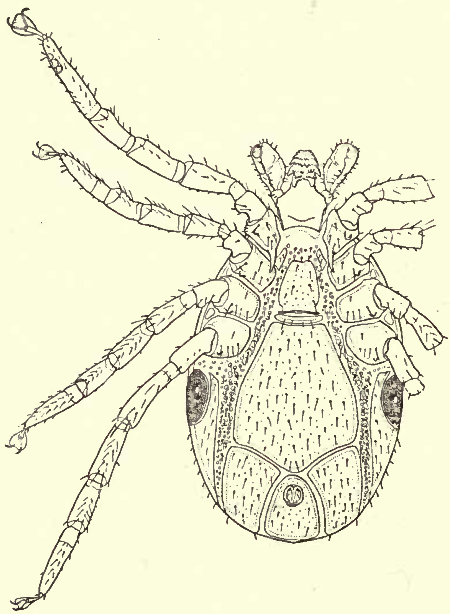

FIG. 89.—*Ixodes ricinus* (L.). Male, seen from the ventral surface. 21 : 1. (After a drawing by A. Dampf.)

The finer structural details will be better seen in cleared specimens and especially in macerated preparations of the chitinous skeleton. The dorsal aspect of the capitulum differs in the two sexes. To right and left, at the base of the female capitulum, is the so-called "porous area," a circumscribed field with numerous pores, not to be mistaken for eyes (Ixodes are eyeless).[2] The distribution of hairs and pores upon the body surface and its appendages should be noted, and the student should then pass on to the study of the legs. The legs are

[1] The ventral surface of the female bears four longitudinal grooves, two of which start near the vulva and run divergently backwards. The other two unite in a curve in front of the anus, and the free ends also run backwards, either parallel to one another or more or less divergent.

[2] In species with eyes, the eyes are always situated upon the scutum.

numbered from the head backwards (I.—IV.), and their joints from the base to the free end (1—6). There is also a nomenclature of the leg-joints, thus : coxa, trochanter, femur, tibia, protarsus and tarsus. At the proximal end of the 3rd and 6th joints, a supernumerary segment is more or less clearly defined, and for this reason many authors reckon eight joints to each limb. The tarsus bears a process which is prolonged into two claws, from the distal end of which hangs a membranous structure, the pulvillum. The hip segments are not attached to the body by means of joints, but are anatomically a part of it. Those which bear the first pair of legs are furnished with a stout thorn, directed backwards, while the tarsi of this pair have a small sense-organ upon the dorsal side.

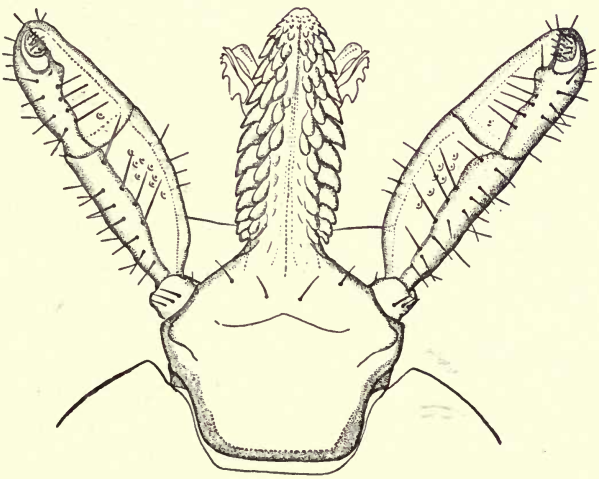

FIG. 90.—*Ixodes ricinus* (L.). Female. Capitulum with mouth-parts, seen from the ventral surface. 57 : 1. (After a drawing by A. Dampf.)

The mouth-parts are twofold, consisting of the hypostome and the cheliceræ. At the sides of the capitulum, externally to the cheliceræ, are the palps. These have four articulations and are characterized by the length of the second and third segments, which are hollowed out upon the ventral surface to form a sort of gutter. The first, or basement joint, is short and the terminal joint is even shorter. The latter proceeds from a terminal hollow in the third joint and is placed at an angle with it, the free end being directed ventrally ; it bears about fifteen small bristles. Between the palps, in the median line, is the rostrum, the most conspicuous feature of which is the hypostome. This is an apparently unpaired organ, placed upon the ventral surface of the rostrum and recognized by many authors as the rostrum itself. Its ventral surface is covered

with rows of recurved teeth, the size, number and arrangement of which differ in the two sexes; the dorsal surface is smooth. Upon the dorsal aspect of the hypostome are the cheliceræ or mandibles. They are surrounded by a sheath which, in the male, is covered with fine transverse lines, but in the female is shagreened. Each mandible consists of a long stem, which is prolonged for a varying distance into the interior of the body, and of a short finger, which bears at its distal end two apophyses, placed externally and internally. The external margin of each is edged with teeth, the number, size and position of which differ, not only in the two apophyses of the same finger, but also, as in the case of the hypostome, in different species and in both sexes.

Fig. 91. — *Dermanyssus hirundinis.* 40 : 1. (After Delafond.)

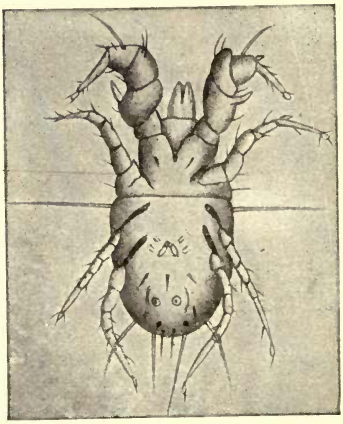

Fig. 92. — *Tyroglyphus farinæ.* Male. 100 : 1. (After Berlese.)

Representatives of other families of the Acarina are readily obtainable. *Dermanyssus hirundinis* (Herm.) is frequently found in swallows' nests, but should not be sought for during the nesting season; *D. gallinæ* (de Geer) is found, though more rarely, in henroosts and dovecots; *Lælaps stabularis* is met with in the litter from cowsheds. These three species belong to the family *Gamasidæ*; they may occasionally attack man. Different species of Tyroglyphidæ are found on cheese, jams, dried fruits, mushrooms, smoked foods, dry anatomical preparations, drugs, and other dry organic substances which are in process of slow decomposition. These species may occasionally attack man, though they are more frequently encountered in vessels employed to hold human secretions and excreta. They have an unstriated cuticle and the legs are furnished with leaf-shaped, unpedunculated suckers. They differ in these two points from the parasitic Sarcoptidæ, Analgidæ, Cytoleichidæ, &c., which otherwise they closely resemble. In the

latter genera the cuticle is transversely striated and the legs are provided with round, pedunculated suckers. The greater number (400) of the species known to us belong to the Analgidæ; they are found on and between the feathers of birds and, occasionally, within the epidermis. Two varieties, *Cytoleichus sarcoptoides*, Mégn., and *Laminosioptes gallinarum*, Mégn., which live in the cellular tissue of the under-skin of fowls, belong to the genus *Cytoleichidæ.* These parasites may penetrate to other organs, where they usually become surrounded by a cystic membrane and, eventually, calcify. They differ from the Sarcoptidæ in the shape of the mouth-parts (which, in Cytoleichidæ, are modified into a sucking tube) and in the longitudinal position of the vulva. Sarcoptidæ live on and in the epidermis of mammals and birds, where they occasion various forms of itch; the mouth-parts are separate and the vulva are placed transversely. Certain species burrow tunnel-like cavities under the epidermis, one end of which communicates with the surface, while the blind end is occupied by the female for the purpose of depositing her eggs. These tunnels may be seen with the naked eye on man; they are up to 3 cm. in length and may be either straight or crooked.[1] Owing to the nature of the skin, they are less readily detected on animals. These burrowing mites belong to the genera *Sarcoptes*, Latr. and *Notœdres*, Raill. They

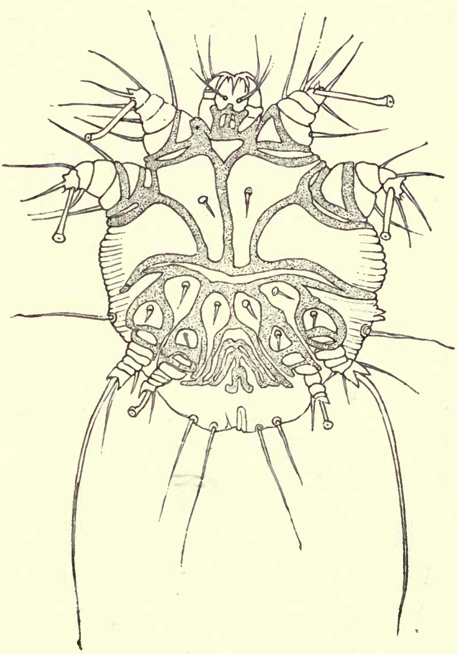

FIG. 93.—*Sarcoptes scabiei.* Male. Ventral aspect. 200 : 1. (After Fürstenberg.)

[1] To obtain specimens for examination, the channel should be opened along its entire length with a finely pointed needle, when the mite will be seen with the naked eye as a shining whitish speck at the end of the tunnel. Or a portion of the epidermis may be cut out with a bent scissors, spread out upon a glass-slide, and cleared with potassium solution or glycerine. The tunnels will be found to contain blackish balls of fæces, eggs in various stages of development, empty egg-shells, and, occasionally, the sloughed cuticle of the female *Sarcoptes*. The six-legged larvæ will not be seen, as, after emerging from the egg, they escape from the tunnel by means of a special opening.

are distinguished from one another by the position of the uterus, which in *Sarcoptes* is terminal, in *Notœdres* dorsal. Other itch-mites of the domestic mammals belong to the genera *Psoroptes*, Gerv., and *Chorioptes*, Gerv. They inhabit the surface of the skin and are usually found among dry crusts and scales. They occur in large numbers and, for this reason, their sexual characteristics and developmental stages are more easily studied than those of *Sarcoptes*.

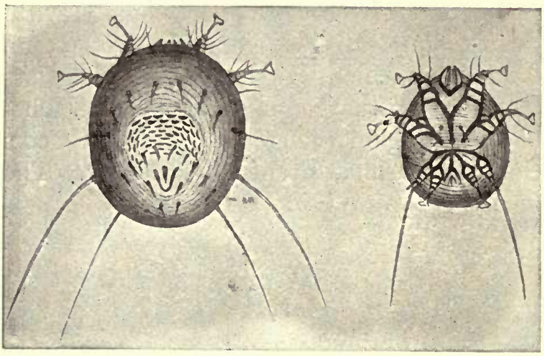

Fig. 94.—*Notœdres cati* (Hering) = *Sarcoptes minor*, Fürstenb. 100 : 1. Left, female from the dorsal aspect; right, male from the ventral aspect. (After Railliet.)

Otodectes cynotis (Hering) = *Chorioptes auricularum* (Raill.) is found in the outer ear, and especially in the cerumen, of dogs and cats.

The hair-follicle mites belong to yet another family, *Demodex*. They inhabit the hair-follicles and sebaceous glands of man and of certain mammals.

All the forms which have been described are sufficiently small to permit of their examination whole, either in the fresh state or after clearing with glycerine. In order to differentiate between species, however, it is sometimes necessary to isolate the chitinous skeleton and its appendages. This is done by very careful maceration in potassium solution in the manner already described.

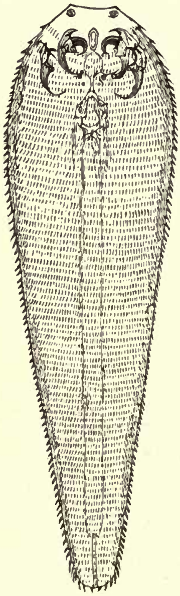

Fig. 95. — The so-called *Pentastoma denticulatum*, being the second larval stage of *Linguatula rhinaria*. 20:1. (After Leuckart.)

Linguatulidæ.

The Linguatulide which is most readily obtainable in Central Europe is *Pentastomum denticulatum*, Rud., the larval stage of *Linguatula rhinaria* (Pilger) (fig. 95). It is found chiefly in the liver, lungs, and mesenteric lymphatic glands of herbivorous mammals, and it may occur, though more rarely, in the carnivoræ and in man. It is a flat, lancet-shaped parasite, 4 to

6 mm. long, with a longitudinal mouth-opening at the anterior end of the ventral surface. This mouth-opening is surrounded by a chitinous ring, and upon either side of it are two claws, each mounted upon a slighter curved basement rod. The body is transparent, the cuticle finely annulated; at the posterior edge of each of the eighty to ninety rings is a fine, recurved tooth. Of the internal organs it is possible to make out the intestinal canal, the genital organs, and sometimes the nervous system. The male and female are distinguished by the position of the genital pore; in the male it is placed upon the ventral surface, in the median line, behind the seventh annulation; in the female it is near the posterior end, immediately in front of the anus. These larval forms when found are frequently dead, the body being in a state of fatty or chalky degeneration. As in the case of calcified cysticerci and echinococci, the portions of the parasite which resist the degenerative process longest are the claws and basement rods. These will be seen if the calcified parasite is treated with some acid solution. The sexually mature parasites inhabit the nasal cavities of the carnivorous and herbivorous mammals. They are present in 6 to 10 per cent. of dogs.

II.—Insecta.

(1) *Bed-bugs* (*Cimex lectularius*).—For purposes of examination specimens should be chosen which have fasted for some time. They should be cleared and, after very careful treatment with potassium solution, they should be examined under the microscope. Insects which are gorged with blood should be kept until the intestinal contents have become absorbed.

The body of the bug is quite flat and is composed of a head-piece, together with three thoracic and eight abdominal segments. The surface, both of the body and of its appendages, is covered with numerous simple, pointed, chitinous hairs and with coarse bristles, one edge of which is serrated. The four-jointed antennæ are situated at each side upon the front of the head; behind them are the eyes, furnished with several strongly arched corneas. Proceeding from the ventral surface of the frontal part of the head is the four-jointed rostrum, which is recurved towards the abdominal surface. The three thoracic segments are quite distinct. The anterior segment, which is also the largest, is deeply incut to allow of the insertion of the head, and has the appearance of a half-moon, with rounded horns directed forwards. The second thoracic ring is smaller than the other two; when viewed from above it is triangular in shape, with the apex pointing posteriorly. At its sides are the scale-like rudimentary wings, which extend backwards as far as the first abdominal

segment. In the middle of the ventral surface, a prolongation of the mesothorax runs back between the hind legs and covers the stink-glands, the secretion from which gives to bugs their characteristic odour.[1] The thin metathorax is almost completely covered by the rudimentary wings.

As in other Insecta, the legs of the bug are divided into coxa, trochanter, femur, tibia, and tarsus. The latter is three-jointed and possesses two claws. The distal end of each tibia is furnished with a bristle.

The abdomen is rounded at the sides and runs off to a point at the back; it is divided into eight segments. The hindermost bears the anus, which is oval in shape, is placed transversely, and is surrounded by a chitinous ring. From it two thin-skinned, tongue-shaped appendages may be protruded, one dorsally and one ventrally. In the male, the hindermost segment also bears the genital opening, which is situated in front of the anus and is associated with the claw-shaped penis, which is usually folded towards the right. The genital opening in the female is a simple longitudinal slit upon the ventral surface of the seventh segment. At the sides of this segment are two thin, wing-shaped processes, directed posteriorly.

All the abdominal segments, with the exception of the eighth, are furnished with two stigmata, placed one upon each side upon the ventral surface; an eighth pair, which is very difficult of detection, is situated upon the anterior edge of the metathorax.

The rostrum, when in repose, is always bent towards the ventral surface; it is a four-jointed tube, tapering towards the free end, within which is another and thinner tube. The outer tube, or sheath, consists of two parts; while the inner tube, or scalpel, is composed of four. The sheath is made up of the labium, or lower lip, and the labrum, or upper lip, while the mandibles and maxillæ go to form the scalpel. It is possible to dissect out these parts, but, owing to their small size in the bed-bug, the manipulation is a difficult one. Among a large number of prepared specimens, however, there will always be one or two in which the component parts of the organ, while remaining attached to the head, are yet separated from one another. It will then be seen that the principal portion of the sheath, formed by the labium, is a long tube, tapering towards the distal end, and in the ventral wall of which, close to the head, is an aperture. This aperture is closed by the labrum, which is modified into a tongue-shaped process.

From the sheath project the divergent tips of the mandibles

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[1] Young insects possess abdominal stink-glands until they slough for the last time. Their thoracic segments, moreover, increase in size from front to back.

and maxillæ. The former are slight structures of unequal length which taper towards their free end, where they are edged with a row of fine teeth. The maxillæ are broader, but they also taper towards the free end; they are of equal length, and are furnished with a small disc-like appendage, placed just in front of the pointed tip. In shape the maxillæ resemble two gutters, the concave surfaces of which face one another and so form a tube. This tube serves to conduct the blood from the wound into the pharynx, and also to convey saliva into the wound.[1] To the sides of and beneath these tubes lie the two mandibles; these are opened during the act of sucking by the agency of several groups of muscles, which proceed from the inner surface of the cuticle of the head and are attached to the dorsal wall of the pharynx. The action of these muscles is counteracted by the elasticity of the cuticle of the pharynx, while the saliva is expelled by means of a syringe of complicated structure, situated ventrally to the pharynx, and with which the ducts leading from the salivary glands communicate.

(2) *Lice.* — Lice live upon the epidermis of warm-blooded animals. They are divided into two groups: Anoploura or Rhynchota aptera, true lice; and Mallophaga, lice which inhabit the appendages of the skin. The latter are furnished with biting mouth-parts and draw their nourishment from horny epidermis cells, hairs and feathers. Of this group the only species of interest is *Trichodectes*, which harbours the Cysticercus of *Dipylidium caninum*, L. True lice are found only upon mammals, whose blood they suck. They are provided with sucking mouth-parts, which are prolonged backwards into the head, but are protruded when in use. Two genera occur on man (*Pediculus* and *Phthirius*) and one is found on the domestic mammals (*Hæmatopinus*).[2]

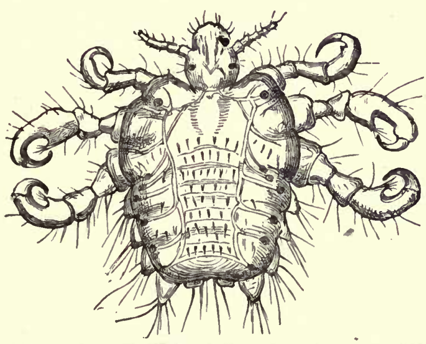

Fig. 96.—*Phthirius pubis.* 30:1. (After Leuckart.)

Phthirius is distinguished from *Pediculus* and *Hæmatopinus*, which

[1] In other Hemiptera, food and saliva are conveyed by two distinct channels, formed by a longitudinal ridge which runs down the groove of each maxilla, dividing it into two parts. Thus, when the maxillæ are closed two parallel tubes are formed, of which the upper leads to the intestine, while the lower one is in communication with the salivary glands.

[2] This family has recently been divided into several groups by Enderlein (*Zool. Anz.*, vol. xxviii, 1905).

are long in shape, by its square, compact body-form. In *Pediculus* and *Phthirius* the eyes are well developed, while in *Hæmatopinus* they are either rudimentary or are absent altogether. In *Hæmatopinus* the sucking organ is longer than in the other two. At the sides of the head are the five-jointed antennæ. The thorax bears upon its under surface three short, powerful legs, the tarsi of which consist of a single piece, modified into a strong, recurved, movable hook. Opposed to this hook, upon the lower end of the tibia, is a thumb-like process which bears a thorn of varying length. In *Phthirius* the first pair of legs is slighter and less formidably armed. The number of abdominal segments is said to differ in each genus, thus: six in *Phthirius,* seven to eight in *Pediculus,* eight to nine in *Hæmatopinus;* but Enderlein believes that all Anoploura have nine abdominal segments. In *Phthirius,* the anterior abdominal segments are crowded closely together, the boundaries being barely recognizable. The posterior segments are flanked by well-marked lateral portions, covered with long hairs. The anal and genital openings are situated at the hinder end of the body, which is pointed in the male but indented in the female. The penis is chitinous and is withdrawn into the interior of the body. There are six pairs of abdominal and one pair of thoracic stigmata.

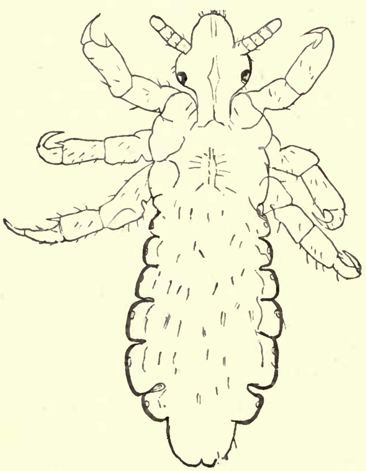

FIG. 97.—*Pediculus capitis.* Male. 40 : 1.

With regard to the mouth-parts of lice, authors differ considerably both in describing the parts and in interpreting their uses. It is certain, however, that, in the act of biting, the anterior portion of the lining of the oral cavity is everted and appears at the front of the head as a blunt barrel- or club-shaped proboscis. The anterior portion of this proboscis is covered with hairs, arranged in rows and directed backwards, which, when the proboscis is withdrawn, lie within the oral cavity. The proboscis, when everted, acts as a sheath through the lumen of which a long, hair-like sting is projected. This sting appears to be homogeneous, but is probably composed of several parts. When not in use the sting is enclosed in a long, tube-like sheath of the same length, which is placed ventrally of the intestine, in the head, and communicates with the latter by means of an opening in the floor of the oral cavity. During the sucking act, which is per-

formed by the pharyngeal muscles, the sting remains projected and serves to conduct the blood into the intestine. The pharynx is prolonged posteriorly into the thin œsophagus, which leads into the sac-shaped stomach, placed in the thorax. At the junction of the stomach and intestine are the openings of the four Malpighian vessels, and six rectal glands open into the hind-gut. Two pairs of salivary glands of different form have been described, one pair being bean-shaped, and one pair horse-shoe shaped.

(3) *Fleas.*—Unlike the bug and the louse, which are flattened dorso-ventrally, the flea is very much compressed laterally; so much so, in fact, that it is almost impossible to prepare whole specimens for the microscope in such a way that they may be viewed from the dorsal or ventral aspect. The body-parts consist of the comparatively small head, which is rounded anteriorly; the thorax, which is divided into three distinct segments; and the abdomen, which is composed of ten segments. On each side of the head is a diagonal groove, and these grooves receive the small, club-shaped antennæ. In front of them in many varieties, including that of man, are the simple eyes, which appear as small, black specks. The mouth-parts are placed in front of, and beneath, the head. They consist of four distinct organs: (1) The long labrum or upper lip, which is grooved upon the ventral surface and is termed variously epipharynx, tongue, or sting; (2) two slender mandibles, also grooved, and bearing several longitudinal rows of teeth; (3) two flat, pointed maxillæ, placed at the sides, and bearing four-jointed palps; (4) the labium or under lip, the two long, jointed palps of which are deeply grooved and unite to form a sheath. This sheath encloses the upper lip and the mandibles, which together form a slender tube. In the sucking act, the tube formed by the hollowed palps of the labium divides, while the inner, or sucking, tube is inserted into the epidermis; blood is pumped up by the action of the well-developed pharynx muscles and flows into the intestine; while at the same time saliva, secreted by four glands situated in the thorax, is conveyed into the wound, partly by way of the hollow groove in the mandibles and partly by way of the labium. The most recent works upon the subject describe a pumping apparatus, into which the salivary ducts of both sides open.

The head is a homogeneous chitinous capsule, but the body segments vary in places both in colour and in thickness. This variation is due to the formation of plates or splints of chitinous material, one of which (tergit) is placed upon the dorsal, and one (sternit) upon the ventral aspect of each abdominal segment. They have the appearance of U or L-shaped bands and are most clearly seen upon the second to seventh segments. The median portion of the splint lies upon the ventral or dorsal ridge, as the case may be,

while the two arms extend along the lateral surfaces. The free, rounded ends of the arms of the dorsal and ventral splints overlap at about the centre of each lateral surface, while the anterior edge of each splint is overlapped by the posterior edge of the splint in front of it. This arrangement permits of considerable distension of the abdomen, such as takes place in all females, and especially in those of *Sarcopsylla,* as a result of the development of the ovaries.

The other abdominal segments, together with their splints, show quite different conditions. There is no ventral splint upon the 1st segment, the ventral splint of the 2nd segment forming the attach-

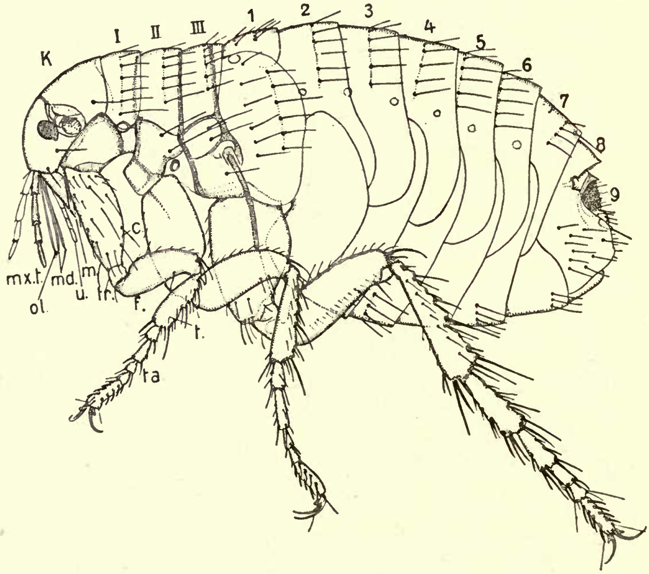

FIG. 98.—*Pulex irritans.* Female. 27 : 1. K, Head, with eye, antenna, and groove of the antenna. I, II, and III, Thoracic segments. 1-9, Abdominal segments (9, pygidium). At the front part of the head, upon its ventral surface, are the mouth-parts : m, maxilla ; md, mandible ; mxt, maxillary palp ; ol, upper lip ; u, lower lip. Behind the mouth-parts is the pleuron of the 1st thoracic segment with the 1st leg : c, coxa ; f, femur ; t, tibia ; ta, tarsus ; tr, trochanter. The legs of the left side only are shown. (After a drawing by A. Dampf.)

ment of the thorax, while the dorsal splint of the first segment is modified into a small triangular or square scale, which meets the 3rd thoracic ring. The splints of the 8th and 9th segments vary according to sex and species. In the male, the dorsal splint of the 8th segment is broad ; it is very much prolonged towards the ventral surface, and extends so far back as almost completely to cover the 9th segment. The ventral splint is proportionately reduced in size, and in the female it is very small indeed.

In both sexes all that is visible of the 9th segment is a small portion of the dorsal splint and the sense-organ or pygidium. The pygidium is an area enclosed in a thick chitinous ring ; its surface is

covered with numerous little thorns and points, between which are single, long sense-hairs, each occupying a round, lighter space. In the male, the lateral arms of the 9th dorsal splint are very much bent towards the ventral ridge, and are continued as straight processes both anteriorly and posteriorly. The two posterior processes bear each a leaf-shaped appendage which is directed backwards, and the free edges of which are set with bristles. This apparatus is employed during copulation to attach the male to the female. The ventral splint of the 9th segment, in the male, takes the form of a two-pronged fork, the prongs of which run towards the dorsal ridge while the handle is directed posteriorly. Between the two prongs is the penis, a chitinous organ, spiral in shape, which is withdrawn into the abdomen. In the female, both the ventral splint and the lateral arms of the dorsal splint are of slight development only; the former is closely covered with bristles.

The 10th segment is rudimentary; upon it is the anus, and below the anus the genital opening.

The dorsal splints of the three thoracic segments are semi-circular in shape, and cover the back and side of each segment. Upon each side of the segment, ventrally of the dorsal splint, is a four-cornered plate, the pleuron, to which the legs are attached. As the pleurons of the 1st thoracic segment are directed forwards and lie under the head, the 1st pair of legs appear to be attached to the head. The pleurons of the 2nd and 3rd thoracic segments are divided by a longitudinal ridge into anterior and posterior halves. The posterior portions of the pleurons of the 3rd thoracic segment are prolonged backwards to the abdomen, and have the appearance of rudimentary wings.

The legs of the flea, like the body, are very much flattened. They increase in length from front to back, and have the same number of joints as in other Insecta. They differ, however, from those of other members of the same group in the remarkable development of the coxæ or thigh-joints, which are as long as the femur and tibia, and are even broader. The five-jointed tarsi, which terminate in two claws, are also comparatively long.

The flea differs from other Insecta, moreover, in possessing a larger number of stigmata, of which there are three thoracic and seven abdominal pairs. The abdominal stigmata are placed upon the lateral surfaces of the 2nd to 8th segments. Two pairs of thoracic stigmata are situated ventrally between the pleurons, while the third is placed dorsally. The head, body segments, and legs are all thickly covered with hairs and bristles, the arrangement of which has considerable specific value. Bristle ridges (rows of thicker bristles) occasionally occur, usually upon the head and thorax.

The abdominal viscera may be obtained in the following manner: The flea should be killed in chloroform and examined under the

microscope upon a glass slide in a drop of normal saline solution. The head is held firmly by means of a needle held in the left hand and inserted into the groove of the antenna. If, with the right hand, a second very fine needle is now inserted diagonally beneath the edge of the 3rd or 4th abdominal segment, the greater part of the abdominal wall may be pulled apart and the viscera will be exposed.

(4) *Diptera*.—Of the larvæ of Diptera, those of Muscidæ are occasionally, those of Œstridæ permanently, parasitic in their habit. These larvæ are commonly known as "maggots," and are readily obtained by exposing a piece of raw meat or the dead body of a small mammal to the air. In summer, decomposition soon sets in, and the smell of putrefaction will attract flies of several different species, which are in search both of food and of a suitable place in which to deposit their larvæ or eggs. The varieties most commonly met with are the blow-fly, *Calliphora vomitoria* (L.), and the very similar *C. erythrocephala* (Meiz.), and the meat-fly, *Sarcophaga carnaria* (L.). The Calliphora varieties deposit eggs, while the Sarcophagidæ are viviparous. The larvæ of the cheese-fly, *Piophila casei* (L.), which perform springing movements, are found in decomposing cheeses of the softer kind; those of *Teichomyza fusca* (Macq.), which are flat in shape and forked at the hinder end, are found in decomposing urine and in earth which has become soaked with it; the larvæ of the house-fly, *Musca domestica*, L., occur principally in dung and other excrements, where the larvæ of other species, notably those of *Homalomyia*, are also usually forthcoming. The larvæ of *Homalomyia* are flat, the segments being furnished upon each side with feathered appendages; they are generally found in rotting animal and vegetable substances.

The rearing of these larvæ artificially is usually a simple matter. The substance containing maggots should be kept upon the ground; it should be kept sufficiently damp; and should be covered over with wire gauze.

The larvæ of *Calliphora*, *Sarcophaga*, and *Musca* are very similar in appearance. The cylindrical body, which is pointed in front and cut off sharply at the back, shows distinct segmentation. At the pointed end, the hard parts of the oral cavity appear as brownish-black marks; the anterior edges of the segments bear circular ridges of a brownish colour; and there are two brown points upon the blunt posterior end. These markings will be clearly seen if the larvæ are killed with hot water or hot 70 per cent. alcohol, and examined, either with a magnifying glass or with a microscope fitted with a low power lens, the object being lighted from above. The dark colour of the segmental ridges is seen to be due to the presence of numerous minute thorns, while the dark points at the posterior end reveal themselves as small oval scales which in, *Calliphora* and *Sarcophaga*, have

three straight fissures, in *Musca* three crooked ones. These fissures are the orifices leading to the tracheæ. The posterior field upon which these stigmata are placed is convex in *Musca*, slightly concave in *Calliphora*, and very much sunken in *Sarcophaga*. In the latter species, its thickened edge (fig. 100, *a*) bears numerous pointed, fleshy growths which are easily seen with the glass. These growths are absent in *Musca*. Ventrally of them in *Sarcophaga* is the anal lamella, which varies in shape in different species.

The stigmatic structures do not remain unchanged during the entire course of larval life; on the contrary, they undergo three developmental changes. In the first stage, when the larva emerges from the egg, both longitudinal tracheal tubes are present, and have their orifices placed posteriorly, as in the older larvæ. The stigma is simpler, however, than in the older larvæ. In *Musca*, it appears as a hole with a heart-shaped border; while in *Calliphora* and *Sarcophaga*, it is a true stigmatic lamella, but furnished with two, not three, fissures. If the external temperature is sufficiently high these larvæ will slough their skin at the end of twenty-four hours, passing into their second developmental stage. Two anterior stigmata, of somewhat different structure, now appear upon either side of the second segment. The longitudinal canals of the tracheal system divide at the body-wall into a fan-shaped arrangement of short branches, which project more or less beyond the body and vary in number and shape according to species. After the first sloughing, the posterior stigmatic apparatus becomes larger, with more clearly defined border and, in the species here described, is generally furnished with two straight fissures. It is not until after the second sloughing that the posterior stigmatic apparatus assumes its final form, that, namely, of three somewhat convergent fissures which, in *Calliphora* and *Sarcophaga*, are straight or very slightly curved, and in *Musca* are serpentine.

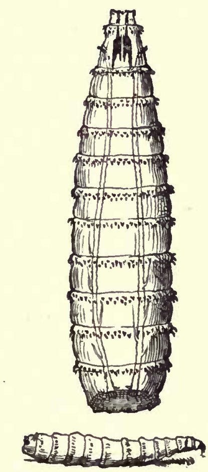

Fig. 99. — Larvæ of *Calliphora vomitoria*. Magnified.

It should be noted in passing that, when the larva sloughs for the third time, the skin is not thrown off, but is retained and, after becoming shorter and very much browner, forms a resistant envelope in which the pupa is enclosed.

Certain changes also take place in the mouth-parts, especially in those of the larvæ of *Musca*. These, as well as certain peculiarities of the chitinized body parts, are best seen in larvæ which have been treated with potassium solution. The action of the fluid will be

hastened if the dead larva is first pricked in the back with a fine needle. After removal from the fluid the soft body contents may be squeezed out through the needle-hole. Clean water should be added and the manœuvre repeated until the body cavity is quite empty. The cuticle and its appendages will now be of sufficient transparency to be examined in water. The soft parts are only to be seen in anatomical preparations.

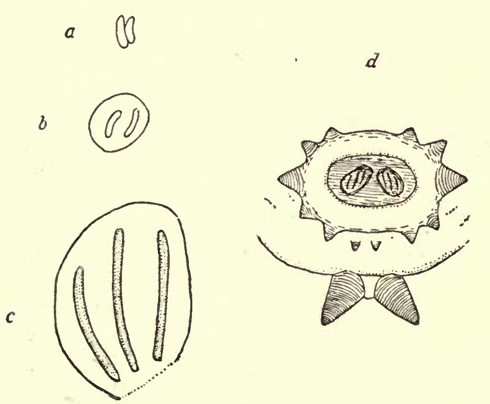

FIG. 100.—*Sarcophaga carnaria.* *a*, *b*, and *c*, The stigmatic apparatus during the three stages of larval development *d*, Anal end of a mature larva seen from behind. Magnified. (After Portschinsky.)

The larvæ of Œstridæ, or Bot-flies, are found in these latitudes in game animals and in the domestic mammals. Three groups are recognized: the *Cuticolæ*, the *Gastricolæ*, and the *Cavicolæ*. The larvæ of several species of *Gastrus* inhabit the intestine of the horse; those of *Hypoderma bovis* are found in abscesses under the skin of cattle; and those of *Œstrus ovis* in the nasal cavities of sheep. As a general rule, only larvæ in the third stage of development are found. Very little information is forthcoming concerning the young stages and, in some cases, they are quite unknown. In certain species the young larvæ inhabit different organs (*Hypoderma bovis*).

The larvæ of Œstridæ are somewhat larger, otherwise they conform to the general structural organization of the larvæ of Muscidæ. Slight differences in the body shape, in the mouth-parts, in the arrangement of bristles upon the body segments, and in the structure of the anterior and posterior stigmata, serve to distinguish between species, without reference to the organ or to the host from which the larvæ have been taken.

Œstridæ may be cultivated artificially in earth, but only if ripe larvæ—those, that is, which are on the point of leaving the host in order to become pupæ—are used. The ripe larvæ are recognized by their colour, which changes before they quit the host.

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